

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE

<b>Transmittal Letter</b>		Docket Number: 1247/1
Patent Number 4,692,435	Issued September 8, 1987	<span style="float: right; font-size: 2em; color: #800000; margin-right: 10px;">RECEIVED</span> <span style="font-size: 1.5em; color: #800000; margin-right: 10px;">MAY 21 1993</span> <span style="font-size: 1.2em; color: #800000; margin-right: 10px;">SPECIAL PROGRAMS OFFICE</span> <span style="font-size: 1.2em; color: #800000; margin-right: 10px;">AC PATENTS</span> <span style="font-size: 1.2em; color: #800000; margin-right: 10px;">COMMERCIAL STAFF</span> <span style="font-size: 1.2em; color: #800000; margin-right: 10px;">COMMISSIONER FOR PATENTS</span>
Invention Title <b>MUCOPOLYSACCHARIDE COMPOSITION HAVING A REGULATORY ACTION ON COAGULATION, MEDICAMENT CONTAINING SAME AND PROCESS OF PREPARATION</b>		Inventor(s) Lormeau, et al.

Address to:  
Commissioner of Patents and Trademarks  
Box Patent Ext.  
Washington D.C. 20231

SIR:

Please find enclosed an Application for Extension of Patent Term Under 35 U.S.C. §156 which is being filed in connection with the above-referenced patent.

Dated: 27 May 1993

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**U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE**

<b>APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156</b>		Docket Number: <b>1247/1</b>	
Application Number <b>726,178</b>	Filing Date <b>4/23/85</b>	Examiner	Art Unit
Patent Number <b>4,692,435</b>	Issue Date <b>September 8, 1987</b>		
Invention Title <b>Mucopolysaccharide Composition Having a Regulatory Action on Coagulation, Medicament Containing Same and Process of Preparation</b>		Inventor(s) <b>Lormeau et al.</b>	
<p><b>Address to:</b>          Commissioner of Patents and Trademarks          Washington D.C. 20231          Box Pat. Ext.</p> <p>Choay, S.A., assignee and owner of the entire 100% interest in U.S. patent 4,692,435 (the "435 patent") submits this request for patent term extension for the '435 patent.</p> <p>(1) The approved product is LOVENOX® enoxaparin, a low molecular weight heparin product, containing a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons as determined by gel permeation chromatography.</p> <p>(2) Regulatory review of LOVENOX® enoxaparin occurred under 21 U.S.C. § 355.</p> <p>(3) the LOVENOX® product received permission for commercial marketing under 21 U.S.C. § 355 on March 29, 1993.</p> <p>(4) The only active ingredient in the LOVENOX® product is enoxaparin. Enoxaparin has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.</p> <p>(5) This application is being submitted by the owner of the '435 patent, Choay S.A., within the sixty day period permitted for submission pursuant to 37 CFR § 1.720(f). The last day on which the application could be submitted is May 28, 1993.</p> <p>(6) The patent for which an extension is being sought is U.S. 4,692,435, issued</p>			

September 8, 1987. The inventors were Jean-Claude Lormeau, Jean Goulay, and Jean Choay. The '435 patent currently expires December 4, 2001.

(7) A copy of the '435 patent is attached hereto as Exhibit A.

(8) A copy of a terminal disclaimer, disclaiming the terminal portion of the '435 patent is attached hereto as Exhibit B. No certificates of correction or reexamination certificates have been issued. ~~No~~ maintenance payments have been made. (RJ 3/19/00)

(9) The '435 patent claims the approved product, methods of using the approved product, and methods of making the approved product. The applicable patent claims and the manner in which each applicable claim reads on the approved product or method of using the approved product follows:

**Claim 18. Heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, which mixture of fractions have improved antithrombotic activity in vivo which is higher than that of heparin and a whole anticoagulation activity lower than that of heparin, and the physiologically acceptable salts thereof.**

LOVENOX® enoxaparin contains heparinic mucopolysaccharide fractions having

- (a) The Lovenox® product package insert indicates that, at minimum, 68% of the LOVENOX® mucopolysaccharides have molecular weights between 2000 and 8000 daltons; no more than 15% have molecular weight greater than 8000 daltons, and no more than 20% have molecular weights less than 2,000 daltons. A study on the LOVENOX® product by Choay indicated that 90% of the mucopolysaccharides had molecular weights between 1900 and 8500 daltons, and fewer than 1% had molecular weights greater than 11,000 daltons, or less than 1600 daltons, as measured by gel permeation chromatography;
- (b) A study on the LOVENOX® product by Choay indicated that
  - (i) roughly half of the mucopolysaccharides had molecular weight between 10333 and 4096 daltons, and roughly half have molecular weight between 4096 and 2050 daltons;
  - (ii) it exhibited a Yin-Wessler of at least 40, namely approximately 240-250 U/mg;
  - (iii) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, namely about 4.36;

- (c) The LOVENOX® product has improved antithrombotic activity in vivo which is higher than that of heparin and a whole anticoagulation activity lower than that of heparin.
- (d) The LOVENOX® mucopolysaccharide fractions are sodium salts.

**Claim 19. The heparinic mucopolysaccharide fractions of claim 18 wherein the lower molecular weight fractions are free of nucleic acids.**

LOVENOX® enoxaparin is substantially free of nucleic acids.

**Claim 21. The heparinic mucopolysaccharides of claim 18 wherein the molecular weight is not in excess of about 8,000 daltons.**

See the comment above on the molecular weight of LOVENOX® mucopolysaccharides.

**Claim 31. The heparinic mucopolysaccharides of claim 18 wherein fractions have a molecular weight range of about 2,000 to about 8,000.**

See the comment above on the molecular weight of LOVENOX® mucopolysaccharides.

**Claim 32. The heparinic mucopolysaccharide fractions of claim 19 which are soluble in an aqueous-alcoholic medium, and insoluble in pure alcohol.**

The LOVENOX® enoxaparin heparinic mucopolysaccharide fractions are soluble in an aqueous-alcoholic medium, and insoluble in pure alcohol.

**Claim 11. A therapeutic composition for controlling thrombosis and decreasing hemorrhaging and of blood hypercoagulation risks which comprises a therapeutically acceptable carrier and heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, and the physiologically acceptable salts thereof, which mixture of fractions have improved antithrombotic activity in vivo which is higher than**

that of heparin and a whole anticoagulation activity lower and slower than that of heparin.

The LOVENOX® product is a therapeutic composition for controlling thrombosis and decreasing hemorrhaging and of blood hypercoagulation risks. It contains a therapeutically acceptable carrier. The composition of the mucopolysaccharide fractions is described above with respect to claim 18.

**Claim 12. The therapeutic composition of claim 11 which is a solution.**

The LOVENOX® product is marketed as a solution.

**Claim 13. The therapeutic composition of claim 12 wherein the heparinic mucopolysaccharides fractions are in solution in a concentration of about 1,000 to 100,000 Yin-Wessler units per ml.**

The LOVENOX® product is marketed in solution. Choay S.A. has determined that the solution has a concentration of roughly 25,000 Yin-Wessler units per mL.

**Claim 14. The therapeutic composition of claim 13 which is a solution of the mucopolysaccharides in a concentration of about 5,000 to about 50,000 Yin-Wessler units per ml.**

The LOVENOX® product is marketed in solution. Choay S.A. has determined that the solution has a concentration of roughly 25,000 Yin-Wessler units per mL.

**Claim 15. The solution of claim 12 which is apyrogenic.**

The LOVENOX® product is apyrogenic.

**Claim 16. The solution of claim 15 which is sterile.**

The LOVENOX® product is sterile.

**Claim 4. A therapeutic method for controlling thrombosis and decreasing blood hypercoagulation and hemorrhaging risks in a patient which comprises administering to the patient in an**

**antithrombotic effective amount, a composition which comprises a therapeutically acceptable carrier and heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, and the physiologically acceptable salts thereof, which mixture of fractions have improved antithrombotic activity in vivo which is higher than that of heparin and a whole anticoagulation activity lower than that of heparin, and said method controlling thrombosis by selectively inhibiting coagulation factor Xa while also having a whole anticoagulation effect which is slower and lower than that of heparin.**

The LOVENOX® therapeutic composition has been approved by the FDA for use in a method for prevention of deep vein thrombosis, which may lead to pulmonary embolism following hip replacement surgery, via injection of LOVENOX® solution. The composition has been described above under claim 18.

**Claim 5. The method of claim 4 wherein the administration is by injection or infusion to the patient.**

The LOVENOX® product has been approved by the FDA for administration by injection.

**Claim 6. The method of claim 5 wherein the administration by injection is sub-cutaneous.**

LOVENOX® has been approved by the FDA for administration by injection. It is not indicated for intramuscular administration. It may be administered sub-cutaneously.

**Claim 7. The method of claim 6 wherein the dosage administered sub-cutaneously is from about 1,000 to about 25,000 Yin-Wessler units per ml.**

The LOVENOX® product is marketed in solution. Choay S.A. has determined that the concentration is roughly 25,000 Yin-Wessler units per mL.

**Claim 47. The therapeutic method of claim 4 wherein the patient is exposed to risks of hypercoagulability.**

LOVENOX® enoxaparin is indicated for patients who are exposed to risks of hypercoagulability.

**Claim 33.** A therapeutic composition which presents less risks than heparin of blood hypercoagulation and of a host hemorrhaging, which composition has improved antithrombotic activity (anti-Xa activity) and improved selectivity with respect to anti-Xa activity than heparin in vivo and a lower and slower anticoagulation activity than heparin, and which composition comprises a therapeutically acceptable carrier and an antithrombotic effective amount of heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, which mixture of fractions have improved antithrombotic activity in vivo which is higher than that of heparin and a whole anticoagulation activity lower than that of heparin, and the physiologically acceptable salts thereof.

The LOVENOX® product is a therapeutic composition which presents less risks than heparin of blood hypercoagulation and of a host hemorrhaging. The composition and properties of LOVENOX® are discussed above with respect to claim 18.

**Claim 35.** The therapeutic composition of claim 33 in which the molecular weight of the heparinic mucopolysaccharides is not in excess of about 8,000 daltons.

See comment (a) to claim 18 for the molecular weight distribution of LOVENOX® enoxaparin.

(10) The relevant dates and information pursuant to 35 USC 156(g) in order to enable the Secretary of Health and Human Services to determine the applicable regulatory review period is:

IND number: 31532  
IND effective date: May 19, 1988

NDA number 20-164  
NDA effective date: December 31, 1991  
NDA approval date: March 29, 1993

(11) The LOVENOX® NDA was approved by the FDA on an IND and NDA filed by Rhone-Poulenc Rorer Pharmaceuticals, Inc. ("RPRP"), the licensee of the '435 patent. As a brief description of the significant activities undertaken by Rhone-Poulenc Rorer Pharmaceuticals, Inc., during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities, attached hereto as Exhibit C is a brief chronology of the communications with the FDA during the regulatory review period ending with approval on March 29, 1993.

(12) In the opinion of the applicant, the '435 patent is eligible for patent term extension under 35 USC 156 because

- (a) 35 U.S.C 156(a)  
The '435 patent claims a product, and a method of using a product.
- (b) 35 U.S.C 156(a)(1)  
The term of the '435 patent has not expired before submission of this application.
- (c) 35 U.S.C. 156(a)(2)  
The term of the '435 patent has never been extended.
- (d) 35 U.S.C. 156(a)(3)  
The application for extension is submitted by Choay S.A., the owner of record in accordance with the requirement of 35 U.S.C. 156(d) and rules of the U.S. Patent and Trademark Office.
- (e) 35 U.S.C. 156(a)(4)  
The LOVENOX® product has been subjected to a regulatory review period before its commercial marketing or use.
- (f) 35 U.S.C. 156(a)(5)(A)  
The commercial marketing or use of the LOVENOX® product, after the regulatory review period is the first permitted commercial marketing or use of LOVENOX® product under the provision of the Federal Food Drug and Cosmetic Act (21 U.S.C. 355) under which such regulatory review period occurred.
- (g) 35 U.S.C. 156(c)(4)  
No other patent has been extended for the same regulatory review period for the LOVENOX® product.

The length of extension of the patent term of the '435 patent claimed by applicant is 887 days, until May 9, 2004. The length of the extension was determined pursuant to 37 C.F.R. 1.775 as follows:

- (a) 1163 The number of days in the period beginning on the date an exemption under subsection (i) of section 505 or subsection (d) of section 507 of the Federal Food Drug and Cosmetic Act became effective for the approved product (May 19, 1988) and ending on the date the application was initially submitted for such product under those sections or under section 351 of the Public Health Service Act (July 26, 1991); (37 C.F.R. 1.775(c)(1))
- (b) 612 The number of days in the period beginning on the date

the application was initially submitted for the approved product under section 351 of the Public Health Service Act, subsection (b) of section 505 or section (507) of the Federal Food, Drug, and Cosmetic Act (July 26, 1991) and ending on the date such application was approved under such section (March 29, 1993). (37 C.F.R. 1.775(c)(2))

- (c) 1775 The sum of (a) and (b). This is the regulatory review period. (37 C.F.R. 1.775(c))
- (d) 0 the number of days in the regulatory review period which were on and before the '435 patent issued (September 8, 1987). (37 C.F.R. 1.775(d)(1)(i))
- (e) 0 the number of days in the regulatory review period during which it is determined under 35 U.S.C 56(d)(2)(B) by the Secretary of Health and Human Services that applicant did not act with due diligence.<sup>1</sup> (37 C.F.R. 1.775(d)(1)(ii))
- (f) 0 the sum of (d) and (e).
- (g) 1775 (c) - (f)
- (g) 887 1/2 of (g)
- (h) 887 (g) minus (f) (37 C.F.R. 1.775(d)(1))
- (i) 12/4/2001 The original term of the '435 patent, shortened by any terminal disclaimer.
- (j) 5/9/2004 The original term of the patent as shortened by any terminal disclaimer plus the number of days in (h). (37 C.F.R. 1.775(d)(2))
- (k) 3/29/2007 The date of approval of the application under section 351 of the Public Health Service Act, or subsection (b) of section 505 or section 507 of the Federal Food, Drug and Cosmetic Act plus 14 years. (37 C.F.R. 1.775(d)(3))
- (l) 5/9/2004 The earlier of (j) and (k). (37 C.F.R. 1.775(d)(4))

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1. There has been no such determination. To the best of applicant's knowledge, RPRP was diligent during the regulatory review period.

- (m) 12/4/2005 (i) plus 5 years. (37 C.F.R. 1.775(d)(5)(i))  
(m) 5/9/2004 The earlier of (l) and (m). (37 C.F.R. 1.775(d)(5)(ii))

(13) The applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services or the Secretary of Agriculture any information which is material to the determination of entitlement to the extension sought.

(14) Please charge the proscribed fee for receiving and acting upon the application for extension pursuant to 37 C.F.R. 1.20(j) to deposit account 11-0600.

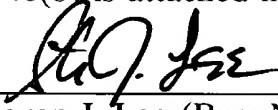
(15) Please address inquiries and correspondence to the undersigned.

(16) A triplicate of these application papers is submitted herewith.

(17) A declaration pursuant to 37 C.F.R. 1.740(b) is attached hereto as Exhibit D.

Dated: May 24, 1993

By:

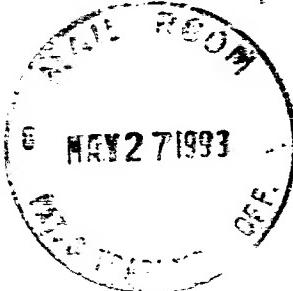
  
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DATE OF DEPOSIT MAY 27, 1993

TYPE OF DOCUMENT APPLICATION FOR EXTENSION OF PATENT TERM FOR  
LORMEAU, ET AL.

PATENT NO. 4,692,435 FILING DATE APRIL 23, 1985

I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 CFR 1.10 ON THE DATE INDICATED ABOVE, BY BEING HANDED TO A POSTAL CLERK OR BY BEING PLACED IN THE EXPRESS MAIL BOX BEFORE THE POSTED DATE OF THE LAST PICK UP, AND IS ADDRESSED TO THE COMMISSIONER OF PATENTS AND TRADEMARKS, WASHINGTON, D.C. 20231.

PHILLIP LIM

(TYPE OR PRINTED NAME OF PERSON MAILING PAPER OR FEE)

Philip Lim  
(SIGNATURE OF PERSON MAILING PAPER OR FEE)

TITLE: MUCOPOLYSACCHARIDE COMPOSITION HAVING A REGULATORY ACTION ON COAGULATION, MEDICAMENT CONTAINING SAME AND PROCESS OF PREPARATION

93 JUN -4 PM 4:00

**United States Patent [19]**

Lormeau et al.

[11] Patent Number: **4,692,435**  
 [45] Date of Patent: \* Sep. 8, 1987

[54] MUCOPOLYSACCHARIDE COMPOSITION HAVING A REGULATORY ACTION ON COAGULATION, MEDICAMENT CONTAINING SAME AND PROCESS OF PREPARATION

[75] Inventors: Jean-Claude Lormeau, Maromme-la-Maine; Jean Goulay, Oissel; Jean Choay, Paris, all of France

[73] Assignee: Choay, S.A., Paris, France

[\*] Notice: The portion of the term of this patent subsequent to Dec. 4, 2001 has been disclaimed.

[21] Appl. No.: 726,178

[22] Filed: Apr. 23, 1985

**Related U.S. Application Data**

[63] Continuation of Ser. No. 204,505, Nov. 5, 1980, abandoned.

**Foreign Application Priority Data**

Nov. 6, 1978 [FR] France ..... 78 31357  
 Jul. 20, 1979 [FR] France ..... 79 18873

[51] Int. Cl.<sup>4</sup> ..... A61K 31/725; C08B 37/10

[52] U.S. Cl. ..... 514/56; 536/21

[58] Field of Search ..... 536/21; 514/56

**[56] References Cited****U.S. PATENT DOCUMENTS**

4,168,377	9/1979	Choay et al. ....	424/183
4,175,182	11/1979	Schmer .....	536/21
4,281,108	7/1981	Fussi .....	424/183
4,303,651	12/1981	Lindahl et al. ....	424/183
4,315,923	2/1982	Takaes et al. ....	424/183
4,486,420	12/1984	Lormeau et al. ....	536/21

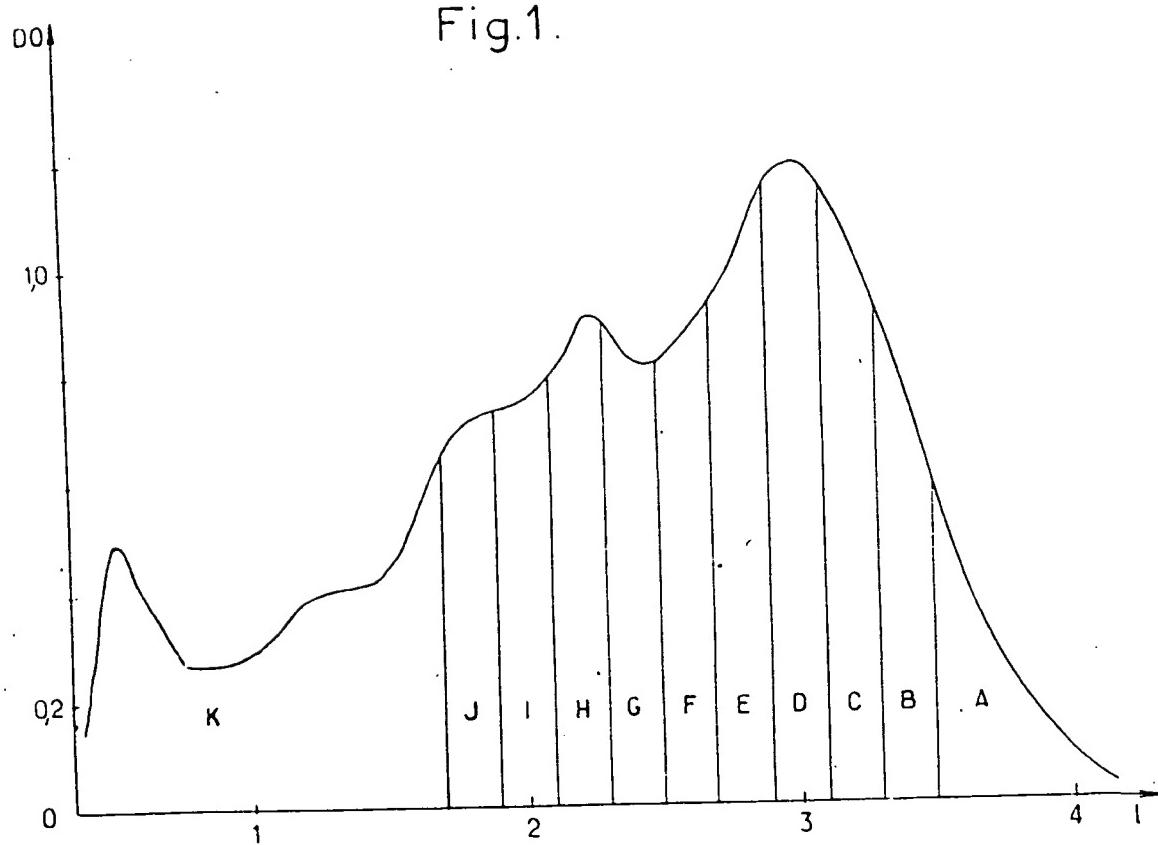
Primary Examiner—Johnnie R. Brown  
 Attorney, Agent, or Firm—Weiser & Stapler

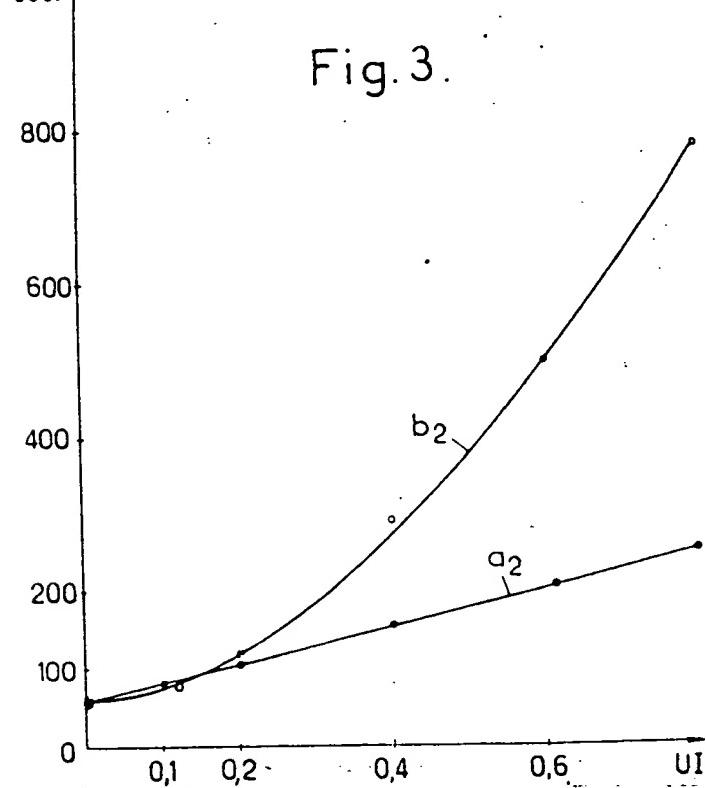
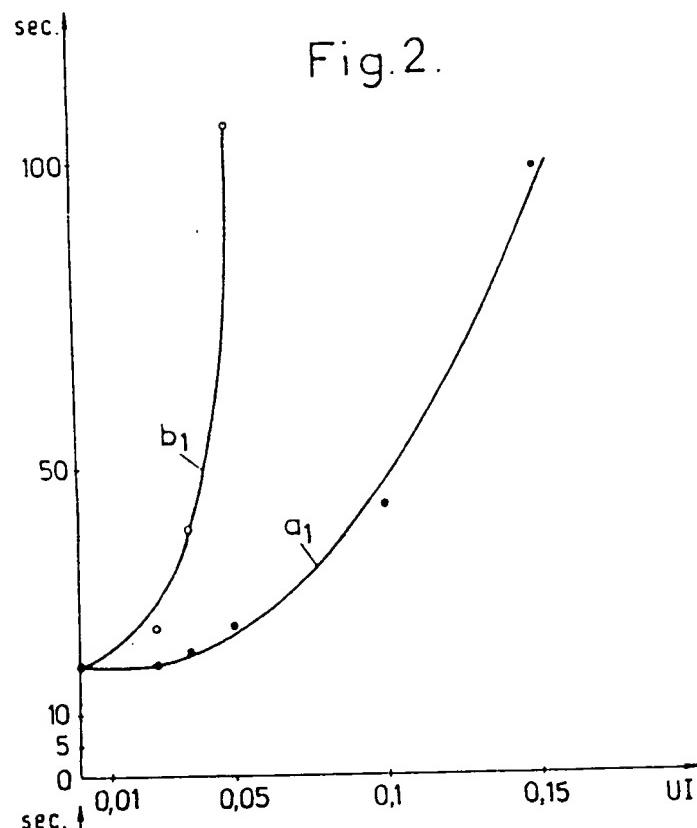
**[57] ABSTRACT**

The invention pertains to a mucopolysaccharide fraction obtainable from heparin or from fractions including heparinic constituents of molecular weights from 2000 to 50,000, which has a Yin-Wessler titer which is high relative to the USP titer. It contains components whose molecular weights are less than 10,000, particularly oligosaccharides in the area of 2000-3000, comprising from 8 to 12, notably 10 monosaccharide units, among which glucosamine units whose primary positions are sulphated. The last mentioned oligosaccharides include one N-acetyl-glucosamine unit per two units of 2-O-sulphate iduronic acid and per two N-sulphate-glucosamine units, the other saccharide units being of a different nature and including distinct substituents.

48 Claims, 15 Drawing Figures

Fig.1.





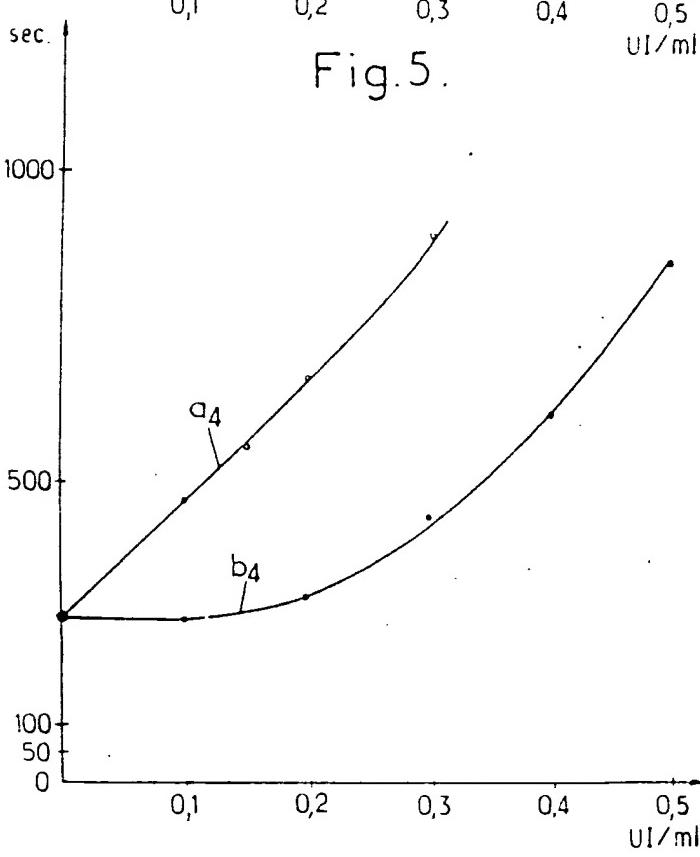
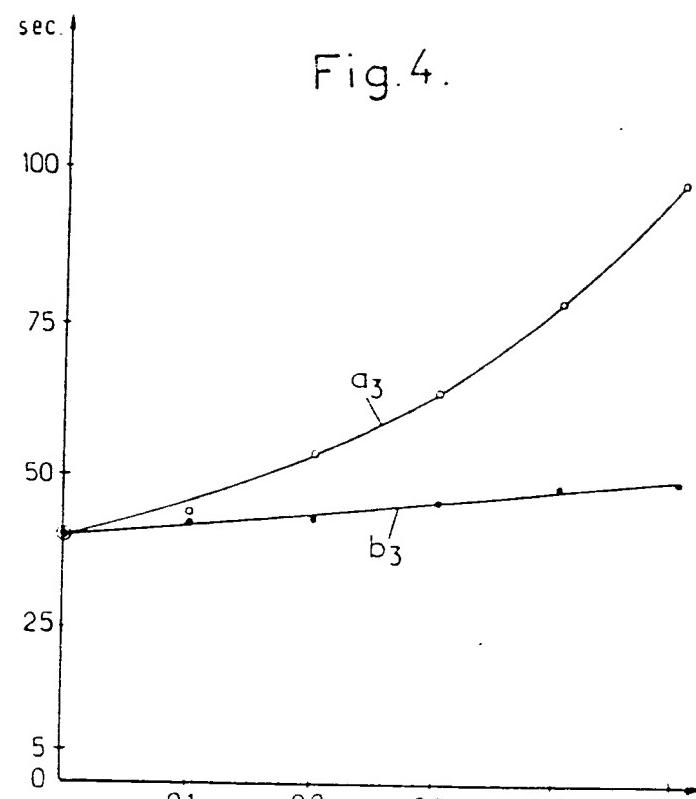


Fig. 6.

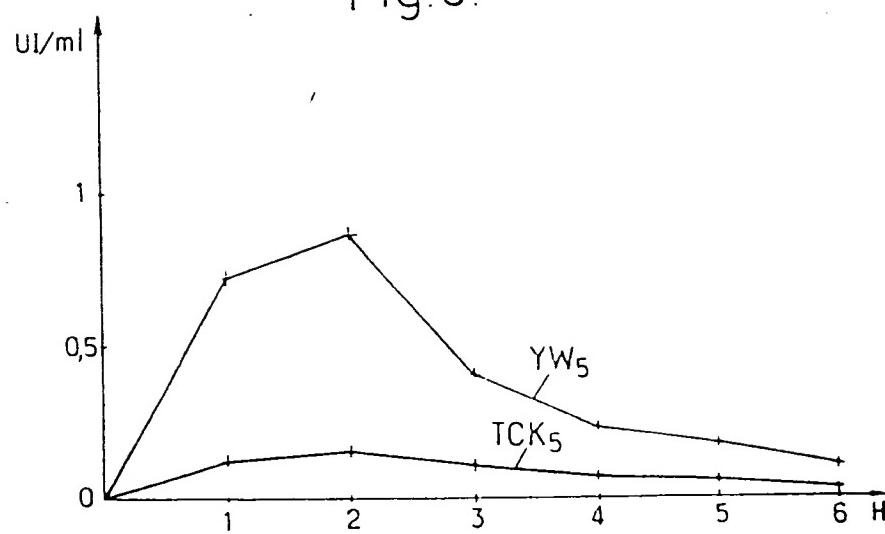


Fig. 7.

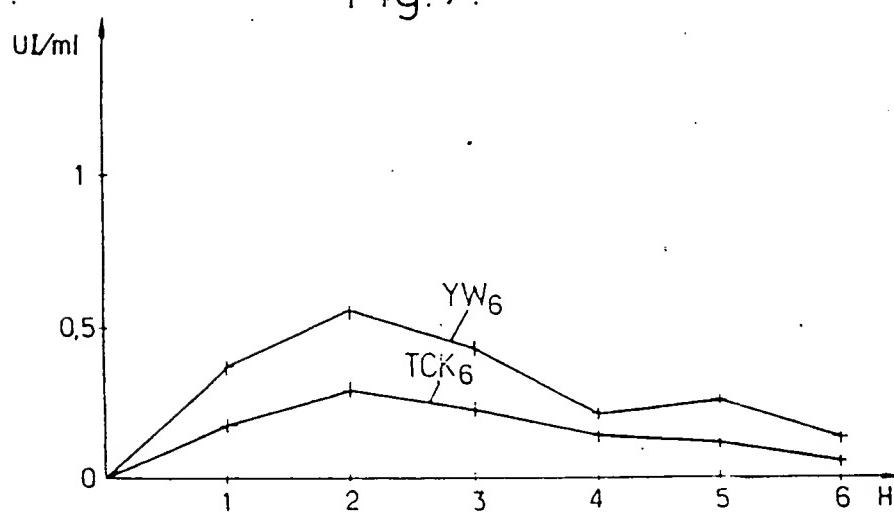


Fig.8.

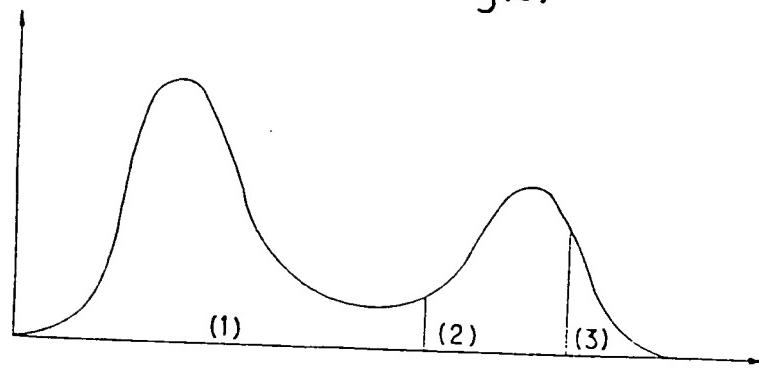


Fig.9.

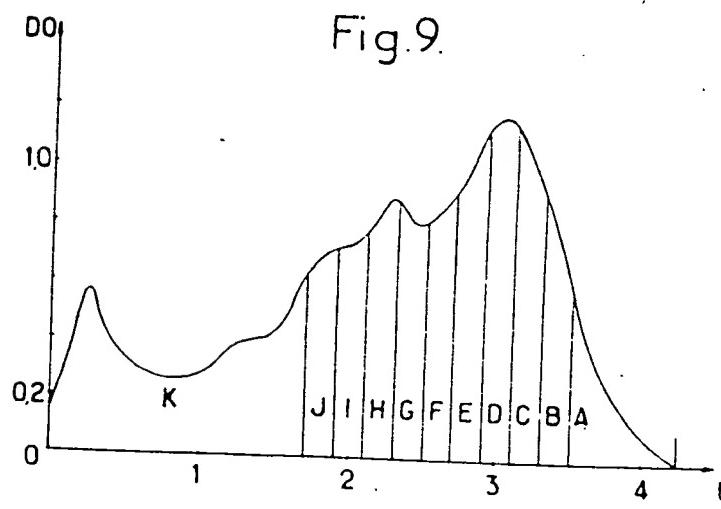


Fig.10.

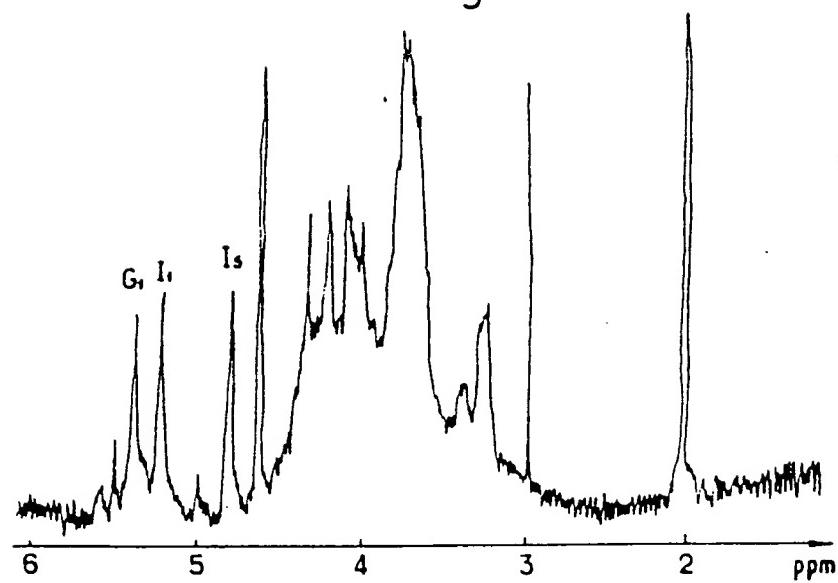


Fig.11.

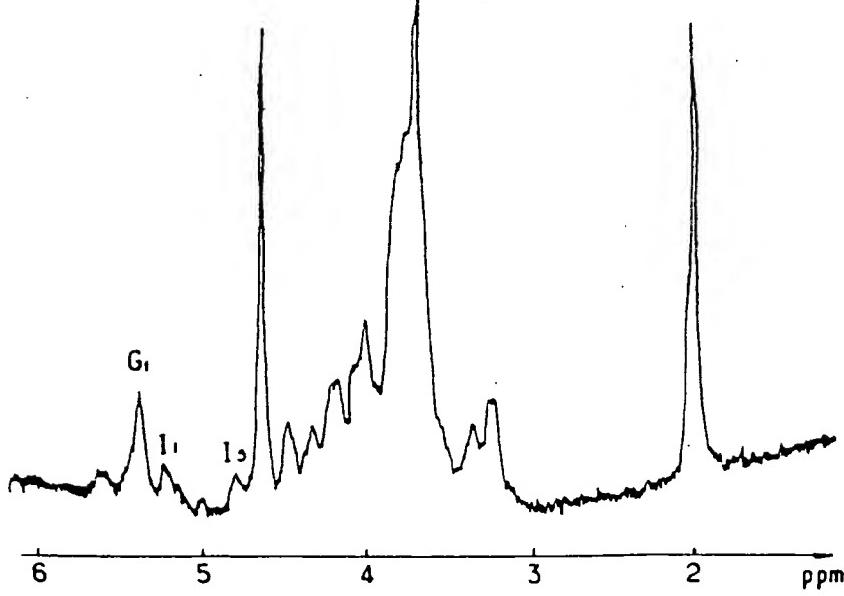


Fig.12

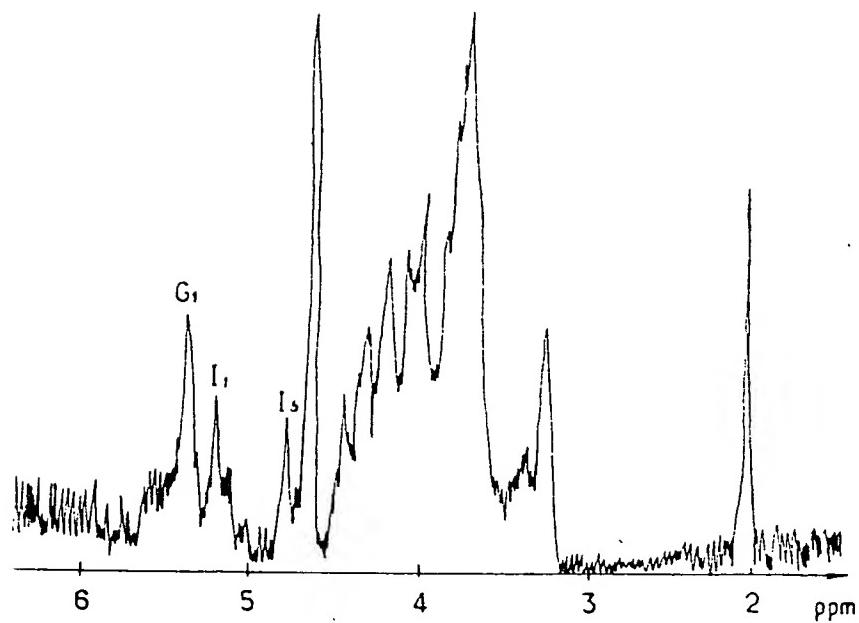


Fig.13.

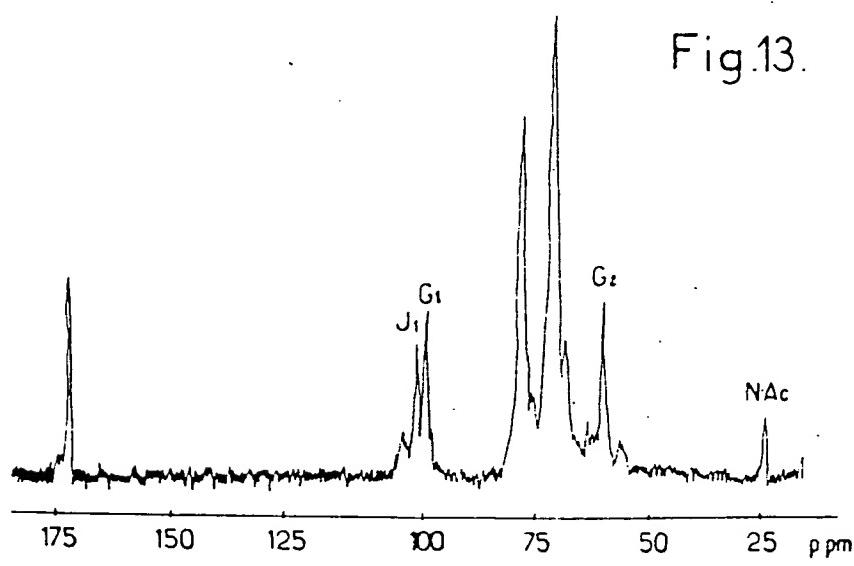


Fig.14.

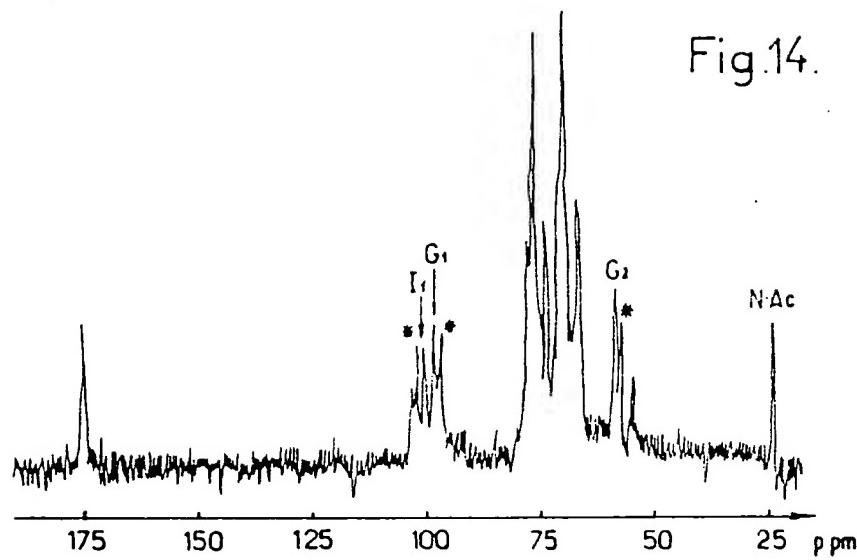
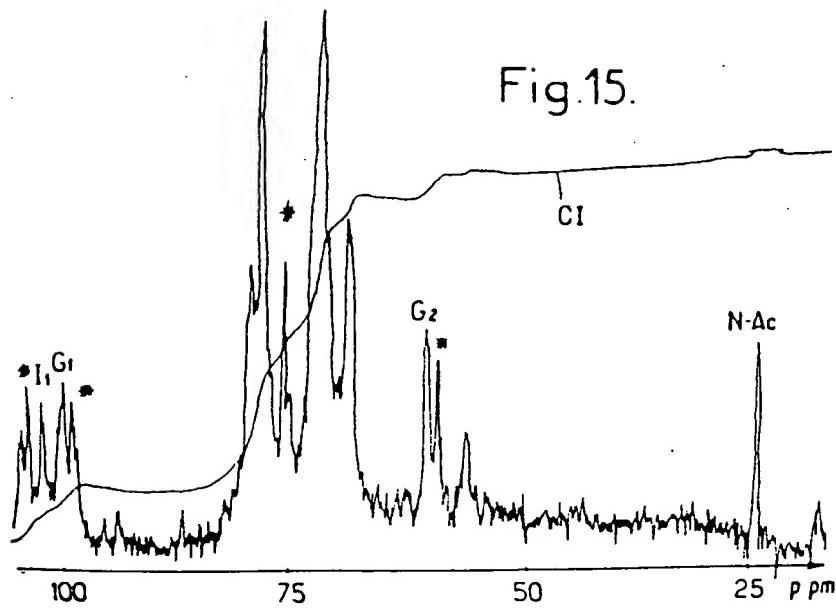


Fig.15.



MUCOPOLYSACCHARIDE COMPOSITION  
HAVING A REGULATORY ACTION ON  
COAGULATION, MEDICAMENT CONTAINING  
SAME AND PROCESS OF PREPARATION

This is a continuation of application Ser. No. 204,505 filed on Nov. 5, 1980, now abandoned.

The invention relates to a mucopolysaccharide fraction endowed with biological properties, enabling it notably to play a regulator role with respect to blood coagulation. Such a fraction can notably be obtained from heparin preparations, such as mammalian tissue extracts.

Certainly heparin is doubtless until now one of the most important anticoagulant medicaments, if not the most important, available to the clinician. It is in fact capable of taking part at several levels in cascades of successive enzymatic reactions, which are normally engaged in the course of physiological hemostasis, in any situation capable of resulting in hypercoagulability of the blood. It is more particularly capable of simultaneously depressing a large number of the coagulation factors participating to the creation and the up keeping of different forms of hypercoagulability.

There will be recalled below, within the limits necessary for clarity of the description, some of the basic notions, purposely expressly simplified, relating to coagulation. The coagulation process comprises in fact three phases generally described as successive, even if they are intrically interrelated:

thromboplastin formation, the phase of prothrombinase (or active thromboplastin) formation,  
thrombin formation, which phase can be summarized as the conversion of the prothrombin into thrombin under the influence of prothrombinase in the presence of ionized calcium and finally,  
fibrin formation, the phase in the course of which the blood fibrinogen is, under the effect of the thrombin, converted into fibrin, which protein tends to become insoluble.

The formation of prothrombinase occurs, in the course of the thromboplastin formation step essentially according to two different routes: the intrinsic or endogenous route, and the extrinsic or exogenous route, which end in the formation of prothrombinases of respectively plasmatic and tissular origins, both capable of activating prothrombin into active thrombin.

The intrinsic or endogenous route (or system) involves a large number of factors or plasmatic proenzymes capable of being successively activated (factors XII, XI, IX, VIII and X), where each activated product (factors XIIa, XIa, IXa, VIIIa and Xa) acts like an enzyme capable of activating the following proenzyme, the activated X factor (Xa) then taking part, notably by reaction with the V factor and a phospholipid of platelet origin, in the production of active endogenous plasmatic prothrombinase. The extrinsic or exogenous system, which can notably be directly dependent of a tissular lesion, calls upon a more limited number of factors and includes notably the production of tissular thromboplastin which, in combination with the VII factor, can, just as the factor VIIIa, convert the inactive X factor into the Xa factor. The activation sequence of the prothrombin into thrombin is then substantially the same as for the intrinsic system, but the phospholipid is here of tissular and not of plasmatic origin.

It is hence possible, to some limited extent to express the idea that the two intrinsic and extrinsic routes join each other at the level of the activation of the X factor (also called Stuart factor), the two following phases of coagulation—thrombin formation and fibrin formation, no longer then giving rise to a distinction between the intrinsic and extrinsic routes.

The outcome of the coagulation process consists in the formation of an insoluble fibrin clot, intended notably to fill in the lesion at the origin of the triggering of this process, for example at the level of a blood vessel.

These coagulation processes normally give rise then to a process, called fibrinolysis, intended to produce lysis of the clot, notably under the effect of plasmin, which enzyme only exists normally in the circulating blood in the form of an inactive precursor, plasminogen, the fibrin itself constituting nonetheless one of the factors capable of initiating the conversion of the inactive plasminogen into fibrinolytically active plasmin.

In fact, although there has, in the foregoing, been presented systems of coagulation and of fibrinolysis as two processes occurring successively in time, it is still not normally so in reality. In fact, there are involved balanced mechanisms, according to extremely complex processes, under the dependence of harmoniously opposed activator and inhibitor factors. The unbalance of these mechanisms, in the sense of hypercoagulability, is then capable of resulting in thromboses. On the other hand, a disequilibrium in the sense of hypocoagulability, exposes the host to hemorrhagic risks.

It is obviously to palliate the effects of hypercoagulability that recourse is currently had to the powerful anticoagulant properties of heparin, in order to bring back the coagulation-fibrinolysis mechanism to equilibrium, each time that the latter is subjected to a considerable disturbance, for example on the occasion of a surgical operation on the host. It is however well known that these attempts at re-equilibration are extremely delicate and that, consequently, the administration of too high a dose of anticoagulant medicament—or the insufficient selectivity of the latter—for the purpose of preventing the risks of hypercoagulation, for example the appearance of post-operative thromboses, may finally be at the origin of serious hemorrhages: whence the necessity of constant watch of the treated patients and of the necessary adjustments of the doses administered—continuously or discontinuously—according to the results of tests, notably of overall coagulability, like the Howell time, which must be practiced at regular intervals.

It is hence an object of the invention to provide active principles of medicaments (and the medicaments themselves) capable of overcoming these difficulties at least in part, notably which are capable of permitting a possible re-equilibration and/or easier control, at the cost of a lesser clinical watch of the coagulation-fibrinolysis system in patients afflicted with a pathology of the coagulation which have undergone a treatment, such as a surgical operation, which expose them to risks of hypercoagulability.

The invention relates more particularly to a mucopolysaccharide fraction exerting a regulator effect with regard to coagulation, notably in causing it to be delayed, yet upon bringing into play inhibitory actions which are more selective than those of heparin, with respect to a smaller number of coagulation factors, more particularly with respect to the activated X factor.

The invention hence relates to a mucopolysaccharide fraction obtainable from heparin or from fractions in-

cluding heparinic constituents of molecular weights extending notably from about 2,000 to 50,000, such as obtained by extraction from mammalian tissues, this fraction being characterized in that it is soluble in an aqueous-alcoholic medium (water-ethanol) having a titer of 55°-61° GL, in that it tends to insolubility in a water-ethanol medium having a higher alcohol content, in that it is insoluble in pure alcohol, and in that it has a Yin-Wessler titer and a USP titer which are respectively in a ratio at least equal to 2, notably at least 3, 10 preferably higher than 6.

These mucopolysaccharide fractions give rise to supplementary fractionations, enabling the preparation of mucopolysaccharide fractions of high specific activity, at the level of the Yin-Wessler titer and having ratios of the Yin-Wessler titer to the USP titer exceeding 10, even 16.

The Yin-Wessler titer is measured by the technique of these authors which is described in "J. Lab. Clin. Med.", 1976, 81, 298-300.

The USP titer, which measures, in manner known in itself, an overall coagulation intensity under well determined conditions, is well known. It is recalled that it is determined in the manner described in the Pharmacopea of the United States XIX, pp. 229-230, (see also the Second Supplement USP-NF, p. 62, and the Fourth Supplement USP-NF, p. 90, respectively entitled "Drug Substances and Dosage Forms".

The invention provides a particularly interesting active principle owing to the capacity that it has of inhibiting the Xa factor in a manner which may be very selective, which capacity contrasts with its activity on the overall coagulation, which may be maintained at a very low level.

This mucopolysaccharide fraction hence constitutes a particularly advantageous anticoagulant medicament active principle, to the extent that it may to this day be admitted that a preferential inhibition of an activated factor, occurring at a stage closer to thrombin formation, practically at the intersection of said intrinsic and extrinsic routes and downstream thereof, is liable of ensuring protection against the risk of hypercoagulability, equivalent to that procured by heparin currently used in therapeutics, without however, by reason of this selectivity of action, resulting in the same hemorrhagic risks as those of conventional heparin. The latter is in effect adapted to inhibit not only the Xa factor, but also other factors coming into play both upstream and downstream of the latter, at other stages of the coagulation routes, for example the factor IIa. It is believed that the re-equilibration in vivo of the coagulation and fibrinolysis system, when the latter tends to become unbalanced under the effect of a pathological cause or of an external operation, for example surgery is easier to achieve with a medicament acting selectively on a specific factor, the factor X, more particularly at the level of inhibition of the factor Xa, than with a medicament capable of acting in un-differentiated manner on several coagulation factors at once.

The invention relates also to a process for obtaining such a mucopolysaccharide fraction, this process comprising:

suspending in an aqueous alcoholic medium of the water-ethanol type, having a titer comprised between about 55° and about 61° GL, preferably of the order of 58° GL, of a substance based on heparin or heparinic constituents whose molecular weights range notably from 2,000 to 50,000, this substance having a low con-

tent of inorganic salts, preferably less than 1% by weight,

separating the insoluble fraction and recovering the solution containing the dissolved mucopolysaccharide fraction, from which it can in its turn be separated, notably by alcoholic precipitation, from the above-mentioned aqueous alcohol medium.

The starting material, from which the mucopolysaccharide according to the invention may be extracted, may be constituted by a heparin of conventional, injectable pharmaceutical quality, or by a crude heparin such as is obtained at the end of extraction operations for this active principle from tissues or organs of mammals, notably from intestinal mucous or from lungs, for example of pork or beef. It can also be constituted by fractions which are normally discarded (waste) in the purification of such crude heparin, for obtaining a heparin of injectable quality and of higher specific activity, provided of course that the waste materials of lower specific activity still contain heparinic constituents.

It is then possible, from raw materials of this type, substantially free from proteins, from nucleic acids and from inorganic salts, preferably when the contents by weight of the latter are less than 1%, to obtain by extraction with 55°-61° GL alcohol a mucopolysaccharide fraction containing constituents of low molecular weight, of which the Yin-Wessler and USP titers are in a ratio of about 2 to about 5, notably from 3 to 5.

It may be remarked that in using water-ethanol mixtures having more than 61° GL, the extraction yield becomes practically zero. On the other hand, the use of aqueous-alcoholic medium of a titer less than 55° GL results in the solubilization of constituents whose presence leads to the lowering of the ratio of the Yin-Wessler/USP titers.

It is to be noted that it is possible to proceed with additional fractionations of the mucopolysaccharide fraction obtained at the end of the above-mentioned process, by various techniques, such as gel-filtration or again selective precipitation in an aqueous-alcoholic medium of predetermined titer, in the presence of proportions also predetermined of an inorganic salt, such as sodium chloride.

An additional fractionation may be achieved by a supplementary step applied to each mucopolysaccharide fraction, previously redissolved in water, which step consists of adding to this aqueous solution from 1 to 2 volumes of ethanol and from 10 to 100 g/l of sodium chloride and of collecting, on the one hand, the equally active precipitate formed and, on the other hand, the content remaining dissolved in the supernatant liquor, notably by a further alcoholic precipitation, and which constitutes a fractionation product whose Yin-Wessler and USP titers respectively are in a ratio still higher, of the order of 6 to 8, than that relating to the initial fraction, notably of the order of 3.

Mucopolysaccharide fractions having a ratio of Yin-Wessler/USP titers which are higher can also be obtained by gel-filtration from the fractions of the first extraction by the 55°-61° GL aqueous-alcohol medium, after prior redissolution of the latter fractions in an aqueous solvent, such as a 0.5M NaCl; 0.1M tris-HCl solution at pH 7.5. Such a solution may be passed through a gel of polyacrylamide and agarose, in bead form, having the tradename ULTROGEL AcA 44, whose effective fractionating zone is situated between effective molecular weights of 4,000 to 60,000 (for linear molecules).

Mucopolysaccharide fractions of the invention, which have a higher Yin-Wessler/USP titer-ratios, are those which flow after the elution of a volume of 2.5 liters, dead volume not included (the dead volume being the volume of liquid contained in the column of gel, notably in the interstitial spaces between the grains of gel), when the gel-filtration is carried out, with a flow rate of 200 ml/hour, in a column having a diameter of 100 mm and a height of 1 m and when the concentration of mucopolysaccharide and the volume of solution placed on the column have been respectively 50 mg/ml and 37.5 ml. The most active fractions are then contained in the 1.5 liters which flow subsequently. The content of the first 2.5 liters is to a great extent formed from heparane-sulphates or heparitine-sulphates, products of high molecular weight and of high viscosity, which do not have anticoagulant activity.

The passage from one column to another column of the same length but of different cross-section entails modification of the volume of solution (of the same concentration) to be placed on the other column, with respect to the volume placed on the preceding column, in a ratio equal to the square of that of the cross-sections (or diameters) of these columns, in order that the same fractions may be obtained in an elution volume from the other column itself also occurring in a ratio with the corresponding elution volume of the preceding column substantially equal to the square of the ratio of said cross-sections.

Gel-filtrations of this type also have the additional advantage, apart from that which resides in the production of fractions in which the ratio of the Yin-Wessler-/USP titers is more favorable, of providing products whose solutions have low viscosity.

In this respect, it should be noted also, that the process according to the invention of extraction of mucopolysaccharide fractions by means of a 55°-61° GL, preferably 58° GL alcohol solution, from a commercial or purified heparin, notably of injectable quality, still containing notably proportions of heparane-sulphates or similar products with high molecular weight, also constitutes in itself a process enabling the reduction in considerable proportions of the viscosity of the aqueous solutions, which can then be formed from these heparins, then essentially free from these mucopolysaccharide fractions.

This reduction in viscosity presents a certain advantage, having regard to the subsequent application of such heparins in anticoagulant therapy, by parenteral, notably sub-cutaneous injection.

From fractions having ratios of Yin-Wessler/USP titers of the order of 6 to 8, it is possible to obtain, by additional fractionations, notably by gel filtration or the like, mucopolysaccharide fractions characterized by ratios of Yin-Wessler/USP titers exceeding 10, notably of the order of 13-16, and having Yin-Wessler titers higher than 130, notably 135 to 160 units/mg.

It is understood that the foregoing indications of molecular weights (and which also follow, notably in the examples) are derived from measurements of the retention time of solutions having a predetermined content of the substance studied, in experiments of gel permeation through a column of gel, under equally predetermined elution conditions, the logarithms of these molecular weight indications being in the same relationship of proportionality with respect to the above-said measurements of retention time, as are those of the molecular weights of 4,000, 6,500, 16,000, 31,000 re-

spectively, of polystyrene-sodium sulphonate standards, notably those marketed by the company named CHROMPACK (Orsay-les-Ulis, France), with respect to their respective retention times, measured in a system and under gel-permeation conditions which are identical.

To the extent where the treated fractions, whatever the degree of purification reached, are in the state of physiologically acceptable metallic salts, such as those of sodium, they may then be converted into mixed or simple salts containing another physiologically acceptable metal, such as calcium, by any process applicable to the salts of heparin. Advantageously, it is possible to resort to the process described in French Pat. No. 73 13580 filed Apr. 13, 1973, by Applicant. It will be recalled that this process consists essentially, starting, for example, from a sodium salt of heparin, of contacting the latter with a different salt of another physiologically acceptable metal, for example calcium chloride, in solution, of then proceeding with the separation of the metallic ions unbound to the heparin (for example by alcoholic precipitation or dialysis) and, to the extent that the substitution ratio reached is not sufficient, of recontacting, in solution, the mixed heparin salt obtained at the end of the first contacting, with a further amount of another salt, notably calcium chloride, according to the desired final substitution ratio.

A further preferred MPS fraction of the present invention can further be obtained from one or the other hereabove described fractions, which further fractions are characterized:

in that, in a gel-filtration operation on a gel column of polyacrylamide and agarose, in bead form, of the type marketed under the name ULTROGEL AcA 44, this fraction flows through after elution of a volume of 2.5 liters, dead volume not included, when the gel-filtration is conducted, at a flow rate of 200 ml/hour, in a column having a diameter of 100 mm and a height of 1 m and when the concentration of the mucopolysaccharide and the volume of the solution placed on the column have been respectively 50 mg/ml and 37.5 ml, the essential of this fraction being notably contained in the 1.5 liters of eluate which then flow through,

by a retention time of the order from 5.7 to 7.5, notably from 6.6 to 7.0 minutes in a gel-permeation system on a column filled with silica of granulometry from 10 to 100 microns, of 250 mm in height and 9 mm diameter, when 50 µl of a solution of 1.3 mg/ml of this fraction in a 0.02M Na<sub>2</sub>SO<sub>4</sub> buffer, having been placed on this column, the elution of said fraction at a flow rate of 3 ml/minute then follows.

Preferred fractions according to the present invention are characterized more particularly again, on the one hand, by a particular affinity with regard to antithrombin III manifested by their capacity to be fixed on the latter, notably in a system comprising the contacting of the fractions with an antithrombin III fixed on a support, such as agarose, in an 0.2M NaCl, 0.05M tris-HCl buffer at pH 7.5 and, on the other hand, by Yin-Wessler and USP titers which are in the ratio (YW/USP ratio) at least equal to 6, the Yin-Wessler titer itself being at least equal to 300 U/mg.

Preferred fractions and compounds according to the invention are characterized by YW/USP ratios higher than 18, with a Yin-Wessler activity higher than 900 U/mg.

Preferably again the fractions and compounds according to the invention are characterized by YW/USP ratios higher than 50.

The preferred compounds of the invention are characterized by YW/USP ratios higher than 65 with a Yin-Wessler activity higher than 1,300 U/mg.

The particular affinity of the fractions according to the invention for Antithrombin III is an essential property to be relied upon for producing such highly enriched fractions, notably from the preceding ones, which process consists of effecting selective fixation of the further enriched fractions or products of the present invention on antithrombin III, notably by contacting the initial fractions with immobilized antithrombin III, particularly on a support, notably agarose, in a buffer such as 0.2M NaCl, 0.05M tris-HCl at pH 7.5, and then eluting the fixed fraction with a buffer of higher ionic force, sufficient to produce desorption, notably a 2M NaCl, 0.05M tris-HCl buffer.

Of course, the starting material from which the last mentioned fractions or compounds are obtainable are not limited to the first fractions according to the invention which have been defined above. Particularly they may be obtained in any other suitable manner, notably from the crude starting material whose nature has been recalled above and from which said first fractions themselves were obtained.

The invention relates more particularly also to the substantially homogeneous compounds and in state of substantial purity which appear to constitute the essential active principle of the preceding fractions.

These compounds are characterized by nuclear magnetic resonance spectra (NMR) carried out under the conditions indicated below and which are the subject of FIGS. 11, 12, 14 and 15.

Referring more particularly to the NMR spectrum of the compounds according to the invention for the proton (<sup>1</sup>H) carried out on solutions of these compounds dissolved in deuteriated water at 35° C. with a radiation of 270 megahertz (MHz) there are observed as characteristic element of the spectrum, resonance signals which, for chemical displacement of the order of 4.8 and 5.2 ppm, are substantially weaker than the resonance signal which is also observed for a chemical displacement of the order of 5.4 ppm (reference for the measurement of the displacements: TSP (sodium 3-trimethylsilyl propionate 2,2,3,3-d<sub>4</sub>)).

The signals observed at the level of the chemical displacements of 5.4; 5.2 and 4.8 ppm correspond to the signals which, in the case of a conventional heparin studied by NMR under the same conditions, are respectively characteristic of the:

- anomer proton, in the 1 position, of the glucosamine N-sulphated units of heparin (signal G<sub>1</sub>);
- anomer proton in the 1 position, of 2-O-sulphated iduronic acid units (signal I<sub>1</sub>) and
- proton in the 5 position of 2-O-sulphated iduronic acid units (signal I<sub>5</sub>).

In conventional heparins, the (G<sub>1</sub>), (I<sub>1</sub>) and (I<sub>5</sub>) signals have all three intensities of the same order of magnitude.

For convenience of language, reference will also be made below, even as regards the fractions or compounds according to the invention, to the signals (G<sub>1</sub>), (I<sub>1</sub>) and (I<sub>5</sub>), to denote the signals observed in relationship with the corresponding chemical displacements (whether for the proton or for <sup>13</sup>C).

This equivalence of language will also extend to the NMR spectrum produced under different conditions and with different references.

Referring more particularly to the NMR spectrum of the compounds according to the invention for carbon 13 (<sup>13</sup>C), effected on solutions of these compounds dissolved in deuteriated water with a radiation of 20 MHz, there are observed as characteristic elements of the spectrum (reference for the measurement of the TMS (tetramethylsilane)):

the absence practically of the resonance signal characteristic of the presence of OH groups on the primary carbon (in the 6 position) of the glucosamine units contained in the mucopolysaccharide fractions of the invention,

additional signals, in the region of the (I<sub>1</sub>) and (G<sub>1</sub>) signals, in regions corresponding to chemical displacements of the order of 100 ppm,

an additional (G<sub>2</sub>) signal close to the G<sub>2</sub>N-sulphate 20 signal in the 60 ppm region,

the presence of a resonance signal in the 75 ppm region (to which normally substantially no resonance signal corresponding in the NMR spectra obtained under similar conditions with a conventional heparin),

25 (the indications of chemical displacements indicated above are estimated with respect to the CH<sub>2</sub> of the N-acetyl-glucosamine groups contained in the MPS according to the invention (25 ppm region in the spectra of the drawings)).

The homogeneous compounds according to the invention, in the practically purified state, which all have the characteristics which have been described already above, as regards their USP and Yin-Wessler activities and their specific affinities for antithrombin III, are also 35 characterized in that they are formed by a homogeneous oligosaccharide having again the following additional characteristics:

it comprises from 8 to 12, notably 10 monosaccharide units;

40 all the primary positions of the glucosamine units of this oligosaccharide are sulphated;

this oligosaccharide comprises one N-acetyl glucosamine unit for two 2-O-sulphate iduronic acid units and for two N-sulphate-glucosamine units, the other saccharides being of different nature and including separate substituents.

The molecular weights of certain at least of the oligosaccharides according to the invention are situated in a range from about 2,000 to about 3,000, notably from 50 about 2,500 where decasaccharides are concerned.

The invention relates also to polysaccharides having the above-indicated general properties, as regards more particularly the USP and Yin-Wessler activities, on the one hand, and the affinity for antithrombin III, on the 55 other hand, these fractions having a higher molecular weight, but also containing in their structure an oligosaccharide part having the above-mentioned structure.

Other characteristics of the invention will appear also in the course of the description which follows of preferred examples of the practising of the invention, notably with reference to the drawings in which:

FIG. 1 shows a characteristic elution diagram of a preferred mucopolysaccharide fraction, according to the invention,

65 FIGS. 2 to 7 show the comparative biological properties of mucopolysaccharide fractions according to the invention and of a conventional heparin with high anti-coagulant activity (in USP titer).

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FIG. 8 is a diagrammatic elution diagram of a fraction according to the invention, on the practising of the process, also according to the invention, of selective separation of said fraction from a fraction also containing other constituents;

FIG. 9 is representative of an elution diagram characteristic of another preferred mucopolysaccharide fraction, according to the invention;

FIG. 10 is the NMR spectrum of a conventional heparin for the  $^1\text{H}$  proton;

FIGS. 11 and 12 are NMR spectra for the  $^1\text{H}$  proton of different fractions according to the invention;

FIG. 13 is an NMR spectrum for the 13 carbon of a conventional heparin used in comparison;

FIG. 14 is an NMR spectrum for the carbon 13 of a fraction according to the invention, and

FIG. 15 is an enlargement of a part of the NMR spectrum of FIG. 14.

#### EXAMPLE I

The raw material was constituted by 100 g of an injectable heparin having a titer of 170 IU/mg (USP units).

To this 100 g of heparin, 2,500 ml of 58° GL alcohol are added. After very vigorous stirring for 15 minutes, 25 vigorous stirring is continued for 15 hours. The suspension is then centrifuged at 7,000 rpm for 1 hour and the supernatant liquor is recovered: 2,400 ml.

To this supernatant liquor 80 ml of saturated sodium chloride solution and then 2,400 ml of 100° GL alcohol 30 are then added.

The precipitated product is recovered, washed with alcohol and dried. It weighs 2.1 g. Its characteristics are as follows:

USP titer: 45 IU/mg

Anti-Xa titer: 160 U/mg.

The anti-Xa/USP ratio was hence 3.55.

#### EXAMPLE II

The raw material used was derived from sub-fractions such as are obtained in the purification of commercial heparin, for the production of injectable heparin. It is obtained notably in part from the supernatant liquor obtained by the addition of 0.6 to 0.7 volume of 100° GL alcohol to an aqueous solution of heparin containing 10 to 20 g per liter of sodium chloride, the precipitated purified heparin then being recovered for purification. The raw material used here also contained various heparin purification residues, notably those obtained by alcoholic precipitations, for freeing injectable heparin from traces of inorganic salts.

To 10 kg of this raw material is added 30 volumes of 58° GL (300 liters) of alcohol. The suspension is subjected to vigorous dispersion and agitation for 15 minutes, the stirring being further maintained energetically 55 for 12 hours. It is then left to stand for 48 hours, in order to produce precipitation of the non-solubilized raw material. The slightly cloudy supernatant liquor is taken up again and clarified by centrifugation.

To the supernatant liquor (volume of 280 liters) is 60 added 10 liters of a saturated solution of sodium chloride, and then 1 volume (280 liters) of 100° GL alcohol. The precipitate obtained, which contains the mucopolysaccharide fraction, is washed with 100° GL alcohol, and then dried.

660 g of a fraction are obtained whose Yin-Wessler and USP titers respectively are already in a ratio higher than 2 (fraction P194HH<sub>(1)</sub>).

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A supplementary fractionation is then made from this fraction, by dissolving the 660 g fraction in 13,200 ml of water.

To the solution formed is added 264 g of sodium chloride, and then 1.5 volumes of 100° GL alcohol (19.8 liters). The precipitated product is collected, washed with alcohol, then dried. 640 g of the P194HH<sub>(2)</sub> fraction are obtained, having the following characteristics:

USP titer: 31 IU/mg,

Yin-Wessler titer: 100 U/mg.

The supernatant liquor contained also active mucopolysaccharide fractions (their recovery is described in Example IV).

The P194HH<sub>(2)</sub> fraction contains also a relatively large amount of substances of high molecular weight mainly heparitine-sulphates, without anticoagulant activity, both in the USP test and in the Yin-Wessler test.

After redissolving in a 0.5M NaCl, 0.1M tris-HCl buffer at pH 7.5, in the proportion of 50 mg/ml, a gel-filtration follows of a volume of 150 ml of the solution on AcA44, in a column of diameter of 215 mm, of 1 meter height, with a flow rate of 800 ml/hour. The high molecular weight substances, of which the major portion is heparitine-sulphates passes in the 10 first liters of eluted solution, dead volume not included.

A mucopolysaccharide fraction with higher Yin-Wessler titer, with a ratio of the Yin-Wessler/USP titers of the order of 4 to 8, can be obtained from the next 6 liters of eluate.

#### EXAMPLE III

This example describes a modification of the processing of the P194HH<sub>(2)</sub> fraction of Example II. After it being dissolved again in a 0.5M NaCl, 0.1M tris-HCl buffer at pH 7.5, in a proportion of 50 mg/ml, it is subjected to a gel-filtration on ULTROGEL AcA 44, in a column of 10 cm diameter, 100 cm height. The elution flow rate was 200 ml/hour.

The eluate was collected in successive fractions of 50 ml. The mucopolysaccharide content of each fraction was evaluated as follows: to 1 ml of the fraction was added 2 ml of 100° GL alcohol. After standing for 2 minutes, the turbidity of the mixture was measured at 660 nanometers, on a spectrometer (optical density measurements). This turbidity was directly proportional to the mucopolysaccharide content of the tested solution.

The C10 fraction, contained in the last third of the fourth liter of eluate, dead volume not included, was collected. The ratio of the Yin-Wessler/USP titers of the C10 fraction was 50/6.

#### EXAMPLE IV

The final supernatant liquor of Example II is itself supplemented with 19.8 liters of 100° GL alcohol and the suspension formed allowed to stand for 24 hours.

The precipitate formed was collected, washed with 100° GL alcohol and dried. 6 g were obtained of a fraction called P194HH<sub>(3)</sub> having the following characteristics:

USP titer: 7 IU/mg,

Yin-Wessler titer: 46 U/mg.

#### EXAMPLE V

The P194HH<sub>(2)</sub> fraction was again dissolved in a 0.5M tris-HCl, 30 g/l NaCl buffer at pH 7.5, in the proportion of 50 mg/ml.

The solution was subjected to gel filtration on an ULTROGEL AcA 44 column (Pharmacia K 100/100, volume: 7 liters; height 100 cm; diameter 10 cm) with a flow rate of 200 ml/hour.

The elution diagram obtained is shown diagrammatically in FIG. 1, showing the variations in the content of material (optical density DO measured at 660 nanometers) as a function of the eluted volume, in liters (1).

There was collected, after passage of a volume of liquid corresponding to the dead volume of the column, successive fractions K, J, I, G, F, E, D, C, B and A, whose volumes are indicated by the length of the corresponding abscissae segments of FIG. 1.

Each of these fractions possess analytical characteristics which are shown in the following table.

TABLE I

Fraction No.	Characteristics of the Fractions Obtained			
	Weight (mg)	USP titer (IU)	Anti-Xa titer (U)	Anti-Xa / USP ratio
A	120	3.7	44.4	12
B	120	4.5	72	16
C	250	6	54	9
D	150	9	135	15
E	300	9	144	16
F	400	11	143	13
G	300	11.5	161	14
H	200	13	143	11
I	50	13	91	7
J	200	7	14	2
K	3500	0	0	/

It is observed that the fractions can be grouped into four types:

(a) The Fractions A, B, C whose elution volumes correspond, in the above-described operational procedure, essentially to the fourth liter eluted, whose USP titers are less than 10 and Yin-Wessler titers less than 80; their molecular weights are at the most of the order of 4,000;

(b) The fractions D, E, F, G, H, whose USP titers are less than 10 and Yin-Wessler titers very high: 135 to 161 units; these fractions also have the most favorable Yin-Wessler/USP titer ratios, from 13 to 16; they are essentially contained in the third liter of eluate; their molecular weights are of the order of 4,000 to 10,000, notably from 4,000 to 8,000;

(c) The fractions I and J, whose ratios of Yin-Wessler/USP titers tend to become unfavorable, and which are probably already contaminated with the K fraction below and

(d) The K fraction, containing again essentially heparane-sulphates devoid of anticoagulant activity.

In Table II are displayed the molecular weights of certain of the fractions estimated according to the retention time measured in gel-permeation, by reference to those of the abovesaid polystyrene-sulphonates of known molecular weight. The fraction F characterized by a main peak corresponding to the retention time of 6.6 minutes and by a shoulder corresponding to a retention time of 6.1 minutes, which testifies to the presence of a constituent whose molecular weight is situated towards 7,200 in the reference system concerned.

The measurements were done by gel-permeation (by means of a Spectraphysics 3500 chromatograph), on columns (250×9 mm) filled with silica of granulometry 10-100 microns, notably those marketed under the name Lichrophospher, of solutions of these fractions in a 0.02M Na<sub>2</sub>SO<sub>4</sub> buffer in the proportion of 1.3 mg of mucopolysaccharide material/ml (volume initially deposited on the column; 50 µm) and with an elution flow

rate of 3 ml/minute. The detection of the material was done by UV spectrophotometry (200 µm).

TABLE II

Product	Retention time (minutes)	Molecular weights relative to polystyrenes
P194III <sub>(a)</sub>	7.0	2,600
P194III <sub>(b)</sub>	6.9	2,900
P194III <sub>(c)</sub>	6.8	3,300
P194III <sub>(f)</sub>	6.6	4,100
"	6.1*	7,200*
polystyrene-sulphonate (1)	6.6	4,000
polystyrene-sulphonate (2)	6.2	6,500
polystyrene-sulphonate (3)	5.4	16,000
polystyrene-sulphonate (4)	4.7	31,000

\*shoulder

## EXAMPLE VI

## Raw Material:

It is formed of byproducts derived from the manufacture of injectable calcium heparinate from crude heparins, such as those extracted from animal tissues (notably intestinal mucous or beef or pork lungs). follows had the following characteristics:

Weight: 252 kg

USP titer: 82 IU/mg

Yin-Wessler titer: 100 IU/mg.

The processing steps described below were then re-sorted to.

## EXTRACTION WITH 58° GL ALCOHOL

The 252 kg of raw material were dispersed in 6,000 liters of 58° GL alcohol with vigorous stirring.

The insoluble phase was separated by decantation and centrifugation.

The soluble fraction was recovered by the addition of NaCl and 100° GL alcohol.

There was obtained:

Insoluble fraction: 230 kg recycled in manufacture,  
Soluble fraction: 20.6 kg (USP titer=21 IU/mg,  
Yin-Wessler titer=90 U/mg).

## THE EXTRACTION OF THE FRACTION OF LOW MOLECULAR WEIGHT FROM THE FRACTION

The fraction soluble in 58° GL alcohol was dissolved in 512 liters of water (20 volumes).

10.24 kg of NaCl were added and then 1.5 volumes of 100° GL alcohol, namely 768 liters. The precipitated fraction was collected, dehydrated with alcohol and dried.

Weight: 19 kg

USP titer: 22 IU/mg

Yin-Wessler titer: 89 U/mg.

This fraction was put aside and subsequently purified and converted into an injectable calcium salt.

The supernatant liquor from the precipitation at 1.5 volumes was supplemented with 1.5 volumes of alcohol, namely 768 liters. The fraction precipitated was collected, dehydrated with alcohol and dried.

Weight: 700 grams

USP titer: 6 IU/mg

Yin-Wessler titer: 40 U/mg.

This fraction of 700 grams was a mixture of low molecular weight MPS, little sulphated high molecular weight MPS and more or less degraded nucleic acids.

#### REMOVAL OF THE NUCLEIC ACIDS FROM THE LOW MOLECULAR WEIGHT FRACTION

The major part of the nucleic acids was removed by precipitation with manganese chloride, in the following manner:

The fraction of 700 grams was dissolved in 7 liters of water. 1 liter of 10% MnCl<sub>2</sub> was added with stirring. The considerable precipitate formed (constituted by the insoluble manganese salts of the RNA and DNA) was removed by centrifugation. The MPS was recovered from the clear supernatant liquor by precipitation with alcohol.

Weight: 480 grams

USP titer: 8 IU/mg,

Yin-Wessler titer: 54 IU/mg.

#### ISOLATION OF THE FRACTIONS OF VERY LOW MOLECULAR WEIGHTS BY GEL-FILTRATION

The very low molecular weights were separated by gel-filtration on ULTROGEL AcA 44.

A column of 200 mm diameter and 1 m height enabled 25 grams to be treated.

The elution diagram is of the type shown in FIG. 8.

Three fractions (numbered from (1) to (3)) were collected. They had the following characteristics (for 25 grams applied at the start):

(1) weight: 16 grams USP titer: 12 IU/mg Y.W. titer: 30 IU/mg,

(2) weight: 7 grams USP titer: 6.5 IU/mg Y.W. titer: 70 IU/mg,

(3) weight: 2 grams USP titer: 2.1 IU/mg y.w. titer: 60 IU/mg.

#### CHROMATOGRAPHY ON INSOLUBILIZED ANTITHROMBIN III

The preceding fraction (3) was subjected to chromatography on antithrombin III fixed on agarose.

A column of 100 ml used at present enabled 700 mg of the fraction (3) to be treated.

The absorption was effected in an 0.2M NaCl, 0.05M tris-HCl buffer at pH 7.5.

The elution was carried out by a 2M NaCl, 0.05M tris-HCl buffer.

The unfixed portion (600 to 650 mg) had a USP titer close to 1 to 2 IU/mg and a Yin-Wessler titer from 10 to 20 IU/mg.

The fixed portion (10 to 30 mg) had a USP titer from 10 to 20 IU/mg and a Yin-Wessler titer from 1000 to 1400 IU/mg.

#### EXAMPLE VII

The fractions A, B and C of Example V hereabove were pooled into a single fraction which was then subjected to an additional fractionation by selective fixation on an agarose-antithrombin III column, under the conditions defined in Example VI.

The fixed fraction was eluted. The fraction named below P194HPA was obtained. It possessed a Yin-Wessler titer of 310 U/mg and a USP titer of 40 IU/mg.

In the same way the above-mentioned fractions E and F for Example V were pooled. The separation procedure of the most active fractions by the technique of fixation-elution defined above, by means of the agarose-

antithrombin III column, was resorted to again. Finally there was obtained a fraction P194HHPF having a Yin-Wessler titer of 900 U/mg and a USP titer of 82 IU/mg.

The said fractions P194HHPA, P194HHPF were subjected to NMR analysis, for the <sup>1</sup>H proton. The same was done with a conventional heparin (7021HH).

The analysis was carried out on each of the said product, previously dissolved in deuteriated water in the proportion of 14 to 62 mg/0.35 ml, with a BRUKER apparatus, 270 MHz, equipped with a FOURIER transform system and enabling the storage of accumulating spectra. The chemical displacements were measured with reference to TSP, as indicated above.

FIG. 10 is representative of the NMR spectrum obtained with conventional heparin. FIGS. 11 and 12 are in the same way representative of the NMR spectra of fractions P 194HHPF and P 194HHPA.

It is noted, by comparison of the NMR spectra, that the (I<sub>1</sub>) and (I<sub>5</sub>) signals of the fractions according to the invention are distinctly less intense than the signal (G<sub>1</sub>), whereas these signals are substantially of the same intensity in the heparin reference spectrum.

#### EXAMPLE VIII

By applying the techniques described in Examples VI and VII to other starting materials, there were obtained similarly fractions:

P 219 HH:

USP titer = 14 IU/mg

Yin-Wessler titer = 1350 U/mg

P 225 HH:

USP titer = 17 IU/mg

Yin-Wessler titer = 1320 U/mg

P 231 HH:

USP titer = 16.2 IU/mg

Yin-Wessler titer = 1400 U/mg

P 194 HH A:

USP titer = 82 IU/mg

Yin-Wessler titer = 900 U/mg

P 242 HH A:

USP titer = 16 IU/mg

Yin-Wessler titer = 1800 U/mg

P 255 HH A:

USP titer = 36 IU/mg

Yin-Wessler titer = 2145 U/mg

The P 242HH A fraction was subjected to NMR analysis, for the <sup>13</sup>C carbon (FIGS. 14 and 15). The same was done with conventional heparin of reference 7071HH (FIG. 13). The analysis was carried out on each of the fractions (in solution in deuteriated water in the proportion of 100 mg in 1 ml of D<sub>2</sub>O with a VARIAN CFT-20, 20 MHz apparatus, equipped with a FOURIER transform system (reference for the measurement of chemical displacements: TMS).

There is observed:

the absence practically of a resonance signal characteristic at the presence of OH groups on the primary carbon (in the 6 position of the glucosamine units contained in the mucopolysaccharide fractions of the invention),

additional signals (not contained in the NMR spectrum of the reference heparin in the region of the (I<sub>1</sub>) and (G<sub>1</sub>) signals, in regions corresponding to chemical displacements of the order of 100 ppm,

a supplementary signal in the 60 ppm region close to the (G<sub>2</sub>) signal,

the presence of a resonance signal in the -75 ppm region (to which normally no resonance signal corre-

sponds in the NMR spectra produced under similar conditions with conventional heparin), (the indications of chemical displacements indicated above are evaluated with respect to the  $\text{CH}_3$  of the N-acetyl glucosamine groups contained in the MPS according to the invention (region of 25 ppm in the spectra of FIGS. 14 and 15).

Signals particular to the fractions or compounds according to the invention are marked with an asterisk in FIGS. 14 and 15.

FIG. 15 also includes the CI integration curve, which enables it to be observed that:

- the compound was homogeneous, hence practically pure,
- it has the characteristics of a decasaccharide,
- it includes one N-acetyl glucosamine unit, for two units of 2-O-sulphate iduronic acid and for two N-sulphate-glucosamine units.

The invention hence enables the preparation of mucopolysaccharide fractions with high anti-Xa activity and having with respect to the Xa factor a remarkable selectivity in the framework of successive enzymatic reactions which characterize the coagulation process.

This remarkable activity and selectivity are also illustrated by the results of the pharmacological tests described below, which were carried out with the P188CH fraction, obtained after the conversion of the P194HHC fraction of Example II, which was in the sodium salt form, into the calcium salt form, by the above-recalled process.

These results are illustrated by the curves of FIGS. 2 to 7, which are all intended to show the comparative anticoagulant effects of the mucopolysaccharide fraction of the invention, on the one hand, and of a conventional heparin (170 USP units/mg), on the other hand.

The curves of FIGS. 2 to 5 are illustrative of the variation observed in vitro of the coagulation times induced in human blood plasmas by increasing doses of a conventional heparin on the one hand, and of the P188CH fraction, on the other hand (the tests corresponding to FIGS. 4 and 5 having been carried out on plasmas free of platelets and consequently impoverished in factor XI).

FIGS. 6 and 7 relate to the comparative results obtained in vivo in the rabbit, with the same P188CH fraction (FIG. 6) and the reference heparin (FIG. 7) (average of the results obtained on groups of five rabbits). Each of the rabbits had received 500 Yin-Wessler units per kg of the composition to be tested.

Concerning firstly FIGS. 2 to 5, they show the variations of the times (in seconds):

- of thrombin (FIG. 2),
- of cephalin-kaolin (FIG. 3),
- of coagulation in the presence of concentrated thromboplastin (FIG. 4) and of diluted thromboplastin (FIG. 5),

induced respectively by the preparation studied, namely the mucopolysaccharide fraction (curves a<sub>1</sub>, a<sub>2</sub>, a<sub>3</sub> and a<sub>4</sub>) and the reference heparin (curves b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub> and b<sub>4</sub>) as a function of the respective doses used, all expressed in USP units/ml.

The thrombin time and the cephalin-kaolin time both constitute types of measurement reflecting rather the action of the preparations studied respectively on the inhibition of the activated factor II and the overall coagulation. The curves of FIGS. 2 and 3 clearly show in this respect that the mucopolysaccharide fraction according to the invention exerts a distinctly lesser effect

than that of the heparin of comparison on the inhibition of the inactivation of a prothrombin and at the level of the overall coagulation. In contrast, FIGS. 4 and 5, which are representative of the phenomena more directly connected with the sequence of enzymatic reactions characteristic of extrinsic coagulation (notably in the relative absence of the factor IIa) show a distinct advantage of the mucopolysaccharide fraction of the invention with respect to the reference heparin. The MPS fraction causes under these conditions a slower coagulation of the blood specimen.

In FIG. 6, there are shown the variations of the activities measured in a rabbit which had received 500 Yin-Wessler units of a mucopolysaccharide fraction of the invention, as a function of time, expressed in hours. To evaluate these activities, recourse is had to the variation of the Yin-Wessler titers (curve YW<sub>5</sub>) and the cephalin-kaolin titers (curve CKT<sub>5</sub>) (IU/ml plasma) as a function of time in hours (H).

The same measurements were carried out with the reference heparin. The corresponding variations of the activities studied are illustrated by curves YW<sub>6</sub> and CKT<sub>6</sub> of FIG. 7.

If FIG. 6 is examined, it is observed that the administration of 500 Yin-Wessler units of the mucopolysaccharide according to the invention causes a considerable anti-Xa activity, compared with the overall coagulability effect, expressed in CKT units, which remains relatively low. It is noted, for example, that at the second hour, the Yin-Wessler activity is 0.85 U/ml, whilst the CKT activity is only 0.15 IU/ml. To the contrary 500 Yin-Wessler units/ml of reference heparin induce an effect expressed by the CKT titers, which is distinctly greater relative to the anti-Xa activity measurable by the Yin-Wessler titer. In particular, it is noted that at the second hour, the anti-Xa activity corresponds to 0.55 U/ml, and that the overall anticoagulant activity, CKT, is of 0.38 IU/ml. The difference between the two titers is hence much smaller than in the case of the mucopolysaccharide according to the invention. The ratio of the Yin-Wessler titer to the CKT titer hence passes from a value less than 2 for the reference heparin to a value greater than 5 for the mucopolysaccharide fraction of the invention.

In vitro and in vivo tests are hence both in the sense of a distinctly more selective action of the mucopolysaccharide fraction of the invention, notably at the level of inhibition of the Xa factor, than that of the reference heparin.

The mucopolysaccharide fractions according to the invention are free of toxicity. The administration of 10,000 U/kg (Yin-Wessler titer) of any of the fractions according to the invention causes in the rabbit neither any toxic reaction nor any pyrogenic effect in the pyrogenicity test in the rabbit according to the French Pharmacopoeia.

The invention hence relates more particularly to mucopolysaccharide fractions of the type which have been described, having notably an activity of at least 40, preferably at least 50, and even more advantageously again of at least 100 U/mg (Yin-Wessler titer). Fractions containing more than 300, particularly more than 900 U/mg (Yin-Wessler titer) are even more preferred. It relates also to pharmaceutical preparations, having similar activities, devoid of pyrogenic substances, and in association with pharmaceutical excipients. It relates in particular to the injectable, sterile, concentrated solutions of these fractions, useful in therapeutics, for the

control of blood coagulation, which solutions contain from 1,000 to 100,000 U (Yin-Wessler)/ml of the mucopolysaccharide fraction, preferably from 5,000 to 50,000, for example 25,000 U/ml, when these solutions are intended for sub-cutaneous injection or containing again, for example, from 5,000 to 10,000, for example 5,000 U units/ml of the mucopolysaccharide fraction, when they are intended for intravenous injection or for perfusion.

The mucopolysaccharide fraction according to the invention is advantageously in the form of a salt of at least one physiologically acceptable metal, such as sodium and/or calcium. Advantageously, these pharmaceutical proportions are presented in the form of syringes usable only once, ready for use at any suitable time.

The compositions according to the invention are particularly adapted to the control (preventive or curative) of the blood coagulation in man or animal, notably in those cases where the host is subjected to risks of hypercoagulability, more particularly those resulting from disturbance of the abovesaid extrinsic phase, for example, as a consequence of the release by the organism of thromboplastin, for example, of tissular thromboplastin (surgical operations, atheromatous processes, tumor development, disturbances of the coagulation mechanisms by bacterial or enzymatic activators, etc.). For the sole purpose of illustrating the invention, and without there being discoverable therein cause for limiting the protection of the invention, there will be indicated below, by way of example, a posology capable of being used in man: it comprises for example, the administration to the patient of 1,000 to 25,000 U by the sub-cutaneous route, 2 to 3 times daily, according to the level of hypercoagulation risk or the thrombotic condition of the patient, or from 1,000 to 25,000 U per 24 hours by the intravenous route, in discontinuous administration at regular intervals or continuously by perfusion, or again from 1,000 to 25,000 U (three times weekly) by the intramuscular route (titers expressed in Yin-Wessler U). The doses should naturally, be adjusted in each patient according to the results of previously effected blood analyses, the nature of the disorder from which the patient is suffering and, generally, his state of health, as is well known.

The invention again also relates to the application of the mucopolysaccharides according to the invention to the constitution of biological reactant usable in laboratory, notably as a comparison reference for the study of other substances of which the anticoagulant activity is to be tested, notably at the level of inhibition of the factor Xa.

As a self-evident and as emerges already from the foregoing, the invention is in no way limited to those of its types of application and embodiments which have been more especially envisaged; it encompasses on the contrary all modifications, in particular those in which the aqueous-alcoholic extraction medium defined above is formed by a mixture of water and an alcohol other than ethanol, for example an aliphatic or aromatic alcohol, preferably cyclic or acyclic saturated aliphatic alcohol, such as primary alcohols including 1 to 6 carbon atoms, it being of course understood that there should be determined in each case, by simple routine operations, the proportions of water/alcohol of the medium which lead to an extraction of a mucopolysaccharide fraction equivalent to that which is obtained with a 55°-60° GL water-ethanol mixture.

Finally it is to be noted that all definitions set forth in the claims that follow are whenever appropriate also part of the present disclosure.

We claim:

1. A process for obtaining heparinic mucopolysaccharides which have improved antithrombotic activity in vivo and inhibition of the Xa-factor (measured in terms of anti-Xa activity) more selective than that of heparin and a lower whole anticoagulation activity than heparin (measured in USP units), which mucopolysaccharides have a molecular weight in the range of about 2,000 to 10,000 daltons, a ratio of anti-Xa to USP titers of at least 3, which process comprises mixing heparin mucopolysaccharides having a molecular weight in the range of about 2,000 to 50,000 daltons in a 55°-61° GL aqueous-alcoholic medium, separating the liquid medium which contains mucopolysaccharides in solution and precipitating out the soluble mucopolysaccharides by alcoholic precipitation, said mucopolysaccharides having an increased ratio of anti-Xa titer to USP titer as compared to the starting heparin mucopolysaccharides.

2. The process of claim 1, which comprises recovering the alcohol-precipitated mucopolysaccharides, subjecting an aqueous solution of said mucopolysaccharides to gel-filtration and recovering the fraction, which fraction has a further increased anti-Xa titer to USP titer ratio as compared to that of the alcohol-precipitated mucopolysaccharides.

3. The process of claims 1 or 2, which comprises the further step of contacting the mucopolysaccharides which have increased anti-Xa titer with antithrombin III, selectively affixing thereon the mucopolysaccharides which have a higher Yin-Wessler activity than the mucopolysaccharides which are not affixed thereon and recovering the affixed mucopolysaccharides by elution, which mucopolysaccharides have further increased anti-Xa titer to USP titer ratio than the starting mucopolysaccharides.

4. A therapeutic method for controlling thrombosis and decreasing blood hypercoagulation and hemorrhaging risks in a patient which comprises administering to the patient in an antithrombotic effective amount, a composition which comprises a therapeutically acceptable carrier and heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, and the physiologically acceptable salts thereof, which mixture of fractions have improved antithrombotic activity in vivo which is higher than that of heparin and a whole anticoagulation activity lower than that of heparin, and said method controlling thrombosis by selectively inhibiting coagulation factor Xa while also having a whole anticoagulation effect which is slower and lower than that of heparin.

5. The method of claim 4 wherein the administration is by injection or infusion to the patient.

6. The method of claim 5 wherein the administration by injection is sub-cutaneous.

7. The method of claim 6 wherein the dosage administered sub-cutaneously is from about 1,000 to about 25,000 Yin-Wessler units per ml.

8. The method of claim 5 wherein the administration by injection is intravenous.

9. The method of claim 8 wherein the dosage administered discontinuously intravenously is from about 1,000 to about 25,000 Yin-Wessler units per ml per 25 hours.

10. The method of claim 4 wherein the administration is intramuscularly in a dosage of from about 1,000 to about 25,000 Yin-Wessler units per ml.

11. A therapeutic composition for controlling thrombosis and decreasing hemorrhaging and of blood hypercoagulation risks which comprises a therapeutically acceptable carrier and heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, and the physiologically acceptable salts thereof, which mixture of fractions have improved antithrombotic activity *in vivo* which is higher than that of heparin and a whole anticoagulation activity lower and slower than that of heparin.

12. The therapeutic composition of claim 11 which is a solution.

13. The therapeutic composition of claim 12 wherein the heparinic mucopolysaccharides fractions are in solution in a concentration of about 1,000 to 100,000 Yin-Wessler units per ml.

14. The therapeutic composition of claim 13 which is a solution of the mucopolysaccharides in a concentration of about 5,000 to about 50,000 Yin-Wessler units per ml.

15. The solution of claim 12 which is apyrogenic.

16. The solution of claim 15 which is sterile.

17. The composition of claim 11 wherein the pharmaceutically acceptable salt is a calcium salt.

18. Heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, which mixture of fractions have improved antithrombotic activity *in vivo* which is higher than that of heparin and a whole anticoagulation activity lower than that of heparin, and the physiologically acceptable salts thereof.

19. The heparinic mucopolysaccharide fractions of claim 18 wherein the lower molecular weight fractions are free of nucleic acids.

20. The heparinic mucopolysaccharides of claim 18 in which the lower molecular fractions have a USP titer less than about 10 units per mg.

21. The heparinic mucopolysaccharides of claim 18 wherein the molecular weight is not in excess of about 8,000 daltons.

22. The heparinic mucopolysaccharides of claim 18 which have a USP titer of about 45 units per mg, a Yin-Wessler titer of about 160 units/mg and a ratio of Yin-Wessler to USP titer of about 3.55.

23. The heparinic mucopolysaccharides of claim 18 in which fractions below 4,000 have a ratio of Yin-Wessler to USP titer which is at least 10.

24. The heparinic mucopolysaccharides of claim 18 in which fractions of about 4,000 have a ratio of Yin-Wessler to USP titer higher than 11, and the Yin-Wessler is at least 900 units per mg.

25. The heparinic mucopolysaccharides of claim 18 in which fractions have a Yin-Wessler to USP titer ratio higher than 60 and a Yin-Wessler of at least 1,300 units per mg.

26. The heparinic mucopolysaccharides of claim 18 wherein fractions above 4,000 have a USP titer not exceeding about 15 units per mg and a Yin-Wessler titer in the range of about 99 to about 160 units per mg.

27. The heparinic mucopolysaccharides of claim 26 wherein the fractions have a ratio of a Yin-Wessler to USP titer is in the range of about 13 to about 16.

28. The heparinic mucopolysaccharides of claim 18 wherein fractions have a USP titer that does not exceed about 6 units per mg, a Yin-Wessler titer not less than about 44 units per mg and the ratio of Yin-Wessler to USP titers if about at least 9.

29. The heparinic mucopolysaccharides of claim 18 having low molecular weight fractions with specific affinity for antithrombin III.

30. The heparinic mucopolysaccharides of claim 18 in which fractions have 8 to 12 monosaccharide units corresponding to a molecular weight ranging from about 2,500 to 3,800.

31. The heparinic mucopolysaccharides of claim 18 wherein fractions have a molecular weight range of about 2,000 to about 8,000.

32. The heparinic mucopolysaccharide fractions of claim 19 which are soluble in an aqueous-alcoholic medium, and insoluble in pure alcohol.

33. A therapeutic composition which presents less risks than heparin of blood hypercoagulation and of a host hemorrhaging, which composition has improved antithrombotic activity (anti-X<sub>a</sub> activity) and improved selectivity with respect to anti-X<sub>a</sub> activity than heparin *in vivo* and a lower and slower anticoagulation activity than heparin, and which composition comprises a therapeutically acceptable carrier and an antithrombotic effective amount of heparinic mucopolysaccharide fractions having constituents of a molecular weight not in excess of about 10,000 daltons, which fractions have (1) a mixture of lower molecular weight fractions in the range of about 2,000 to about 4,000 daltons with higher molecular weight fractions of a molecular weight in the range of about 4,000 to about 10,000 daltons, (2) a Yin-Wessler of at least 40, and (3) a ratio of Yin-Wessler to USP titer in the range of 3 to 5, which mixture of fractions have improved antithrombotic activity *in vivo* which is higher than that of heparin and a whole anticoagulation activity lower than that of heparin, and the physiologically acceptable salts thereof.

34. The therapeutic composition of claim 33 in which the lower molecular fractions of the heparinic mucopolysaccharides have a USP titer less than about 10 units per mg.

35. The therapeutic composition of claim 33 in which the molecular weight of the heparinic mucopolysaccharides is not in excess of about 8,000 daltons.

36. The therapeutic composition of claim 33 in which the heparinic mucopolysaccharides have a USP titer of about 45 units per mg, a Yin-Wessler titer of about 160 units/mg and a ratio of Yin-Wessler to USP titer of about 3.55.

37. The therapeutic composition of claim 33 in which fractions of the heparinic mucopolysaccharides below

4,000 have a ratio of Yin-Wessler to USP titer which is at least 10.

38. The therapeutic composition of claim 33 in which heparinic mucopolysaccharides have fractions of about 4,000 which have a ratio of Yin-Wessler to USP titer higher than 11, and the Yin-Wessler is at least 900 units per mg.

39. The therapeutic composition of claim 33 in which fractions of the heparinic mucopolysaccharides have a Yin-Wessler to USP titer ratio higher than 60 and a Yin-Wessler of at least 1,300 units per mg.

40. The therapeutic composition of claim 33 wherein fractions of the heparinic mucopolysaccharides above 4,000 have a USP titer not exceeding about 15 units per mg and a Yin-Wessler titer in the range of about 99 to about 160 units per mg.

41. The therapeutic composition of claim 40 wherein the fractions of the heparinic mucopolysaccharides have a ratio of a Yin-Wessler to USP titer is in the range of about 13 to about 16.

42. The therapeutic composition of claim 33 wherein fractions of the heparinic mucopolysaccharides have a USP titer that does not exceed about 6 units per mg, a 25

Yin-Wessler titer not less than about 44 units per mg and the ratio of Yin-Wessler to USP titers if about at least 9.

43. The therapeutic composition of claim 33 wherein the heparinic mucopolysaccharides have low molecular weight fractions with specific affinity for antithrombin III.

44. The therapeutic composition of claim 37 wherein fractions of the heparinic mucopolysaccharides have 8 to 12 monosaccharide units corresponding to a molecular weight ranging from about 2,500 to 3,800.

45. The therapeutic composition of claim 37 wherein fractions in the heparinic mucopolysaccharides have a molecular weight range of about 2,000 to about 8,000.

46. The therapeutic composition of claim 37 include the heparinic mucopolysaccharide fractions which are soluble in an aqueous-alcoholic medium, insoluble in pure alcohol.

47. The therapeutic method of claim 4 wherein the patient is exposed to risks of hypercoagulability.

48. The therapeutic method of claim 4 wherein the heparinic mucopolysaccharides have a USP titer of about 45 units per mg, a Yin-Wessler titer of about 160 units/mg and a ratio of Yin-Wessler to USP titer of about 3.55.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of :  
JEAN CLAUDE LORMEAU ET AL : Group Art Unit: 125  
Serial No 204,505 : Examiner: J.R. Brown  
Filed: November 6, 1980 : 703-557-3920  
For a Patent for :  
MUCOPOLYSACCHARIDE COMPOSITION :  
HAVING A REGULATORY ACTION :  
ON COAGULATION, MEDICAMENT :  
CONTAINING IT AND PROCESS :  
FOR PREPARING IT : January 31, 1984

TERMINAL DISCLAIMER UNDER 37 CFR 1.321(b)

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

Choay S.A. of 48 Theophile Gautier 75782 Paris, Cedex 16 (France) the owner of record of application Serial No. 204,505 filed November 6, 1980 and of application Serial No. 301,611 filed September 14, 1981, as evidenced by the assignments recorded under Reel 3707 Frame 791 and Reel 3932 Frame 304, respectively, does hereby disclaim the terminal part of any patent granted on application Serial No. 204,505 which would extend beyond the expiration date any patent granted on application Serial No. 301,611, if the latter patent is granted first.

Choay S.A. hereby agrees any patents so granted on said application shall be enforceable only for and during such period that the legal title to said patents shall be the same.

This agreement shall run with any patent(s) granted on  
the above-said application and shall be binding upon the  
grantee, its successors or assigns.

CHOAY S.A.

by P. Willaime  
Title; General Manager  
P. Willaime

Rhône-Poulenc Rorer Central Research  
Regulatory Affairs

APPLICATION CHRONOLOGY REPORT  
Report Cover Page

Run Date: 05/05/93  
User: ingress\_prod

Selection Criteria

App Number:  
20164

Exhibit C

Rhône-Poulenc Rorer Central Research  
Regulatory Affairs

Page: 1

**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      Type: NDA      Drug Code: RP 54563      Trade Name: LOVENOX INJECTION  
 APP Num: 20164      Dosage Form: SOLUTION  
 Route of Admin.: SU  
 Generic Name: enoxaparin      3.0 mg

COMM DATE	COMM TYPE	CONTENT Name/Description		Comments
		Details	Comments	
26-JUL-91	ORIGINAL SUBMISSION	CLINICAL PRO#: INV NAME:	STUDY	
05-AUG-91	GENERAL CORRESP FROM FDA	OTHER	N/A	FDA ACKNOWLEDGED RECEIPT OF ORIGINAL NDA ON 29-JUL-91
20-SEP-91	GENERAL CORRESP FROM FDA	OTHER	N/A	FDA COMMENTS AFTER PRELIMINARY REVIEW OF ORIGINAL NDA SUBMISSION
15-OCT-91	GENERAL CORRESP TO FDA	OTHER	N/A	CHANGE OF ADDRESS
01-NOV-91	AMENDMENT	OTHER	N/A	RESPONSE TO 20-SEP-91 FDA LETTER INDICATING REFUSAL TO FILE. INFO FOR DISCUSSION AT 13-NOV-91 INFORMAL CONFERENCE
15-NOV-91	MEETING MINUTES	OTHER	N/A	INFORMAL CONFERENCE TO DISCUSS THE REASONS THE NDA WAS NOT ACCEPTED FOR FILING
10-JAN-92	PHONE CALL	PRECLINICAL STUDY#: SPECIES: RTE. ADMIN: DURATION:	STUDY	MS. COLLIER RELAYED THE CSO'S QUESTIONS RE: THE RESUBMISSION
14-JAN-92	GENERAL CORRESP FROM FDA	OTHER	N/A	FDA ACKNOWLEDGED RECEIPT OF RESUBMITTED NDA ON 31-DEC-91. NEW DUE DATE IS 28-JUN-92
21-JAN-92	PHONE CALL	OTHER	N/A	CONSUMER SAFETY OFFICER REQUESTED ADDITIONAL COPIES OF CERTAIN VOLUMES
22-JAN-92	GENERAL CORRESP TO FDA	OTHER	N/A	PROVIDED A NEW COPY OF THE SAS DATASETS OF EFFICACY & BLEEDING ASSESSMENT DATA FOR HIP REPLACEMENT SURGERY
24-JAN-92	GENERAL CORRESP TO FDA	OTHER	N/A	PROVIDED DESK COPIES OF VOLUMES 77 AND 78

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**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      App Num: 20164      Type: NDA      Drug Code: RP 54563      Trade Name: LOVENOX INJECTION  
Route of Admin.: SU      Dosage Form: SOLUTION      Generic Name: enoxaparin      30 mg

COMM DATE	COMM TYPE	CONTENT Details	Name/Description	Comments
28-JAN-92	GENERAL CORRESP To FDA	OTHER	N/A	PROVIDED FDA WITH A DESK COPY OF VOLUME 2.3
30-JAN-92	AMENDMENT	PRECLINICAL STUDY#: SPECIES: RTE. ADMIN:	STUDY	RESPONSE TO THE QUESTIONS DR. AHMAD RAISED DURING HIS REVIEW OF NONCLINICAL DATA
04-FEB-92	PHONE CALL	CLINICAL PRO#: PAT NUM: PAT INIT: PAT REACT: SRTYPE: Follow-up INV NAME:	SAFETY REPORT	CSO BRONNIE COLLIER STATED A SAFETY UPDATE WOULD NOT BE REQUIRED UNTIL THE APPLICATION APPROACHES APPROVABILITY
24-FEB-92	AMENDMENT	OTHER	N/A	FOUR APPENDICES CONTAINING INFO REQUESTED BY FDA
24-FEB-92	GENERAL CORRESP From FDA	CMC DOS Form/Potency: INJECTION	DRUG PRODUCT	FDA'S RESPONSE TO STABILITY DATA
18-MAR-92	PHONE CALL	CMC DOS Form/Potency:	DRUG PRODUCT	RESPONSE TO 18-MAR-92 REQUEST FOR TWO DISKETTES OF PROPOSED PACKAGE INSERT
19-MAR-92	AMENDMENT	LABEL/PRO MATERIAL FC NUM:	PACKAGE INSERT	REQUEST FOR INFORMATION RE: STABILITY DATA FOR EXPIRATION DATING
19-MAR-92	GENERAL CORRESP From FDA	CMC DOS Form/Potency:	DRUG PRODUCT	RESPONSE TO 24-FEB-92 REQUEST FOR FURTHER INFORMATION
26-MAR-92	AMENDMENT	CMC DOS Form/Potency:	DRUG PRODUCT	RESPONSE TO FDA REQUEST FOR INFORMATION RE: CLINICAL STUDY ENO 884
31-MAR-92	AMENDMENT	OTHER	N/A	RESPONSE TO 31-MAR-92 REQUEST FOR INFORMATION
01-APR-92	AMENDMENT	CLINICAL PRO#: 526 INV NAME:	STUDY	RESPONSE TO 31-MAR-92 REQUEST FOR INFORMATION

**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      NDA      Drug Code: RP 54563      Trade Name: LOVENOX INJECTION  
 APP Num: 20164      Type: GENERAL CORRESP      Dosage Form: SOLUTION  
 Route of Admin.: SU      Generic Name: enoxaparin      30 mg

COMM DATE	COMM TYPE	CONTENT Details	Name/Description	Content Comments
03-APR-92	GENERAL CORRESP From FDA	OTHER	N/A	31-MAR-92 SUBMISSION CONSIDERED MAJOR - EXTENDED DUE DATE TO 27-AUG-92
06-APR-92	AMENDMENT	CMC Dos Form/Potency:	DRUG PRODUCT	RESPONSE TO RECENT TELEPHONE REQUEST FOR SAMPLES OF SYRINGES AND BLISTER PACKAGES
08-APR-92	AMENDMENT	CMC Dos Form/Potency:	DRUG PRODUCT	RESPONSE TO 19-MAR-92 FDA REQUEST FOR ADDITIONAL STABILITY INFORMATION TO SUPPORT PROPOSED EXPIRATION DATING
08-APR-92	GENERAL CORRESP To FDA	OTHER	N/A	RESPONSE TO 02-APR-92 REQUEST FOR SPECIFIC INFO TO FACILITATE THE UPCOMING CLINICAL INVESTIGATOR INSPECTIONS IN CANADA
10-APR-92	GENERAL CORRESP To FDA	OTHER	N/A	ADDITIONAL INFO REQUESTED 02-APR-92 RE: CASE REPORT FORMS FOR ENO884 AT HAMILTON GENL HOSP & HENDERSON GENL HOSPITALS
17-APR-92	GENERAL CORRESP To FDA	OTHER	N/A	CONFIRMATION OF FDA INSPECTION DATE OF CLINICAL TRIAL ENO884 AT HENDERSON & HAMILTON HOSPITALS
21-APR-92	GENERAL CORRESP To FDA	OTHER	N/A	RESPONSE TO 14-APR-92 FDA TELEPHONE CALL RE: PRE-APPROVAL INSPECTIONS FOR JULY OF 1992
01-MAY-92	GENERAL CORRESP To FDA	CMC Dos Form/Potency:	DRUG PRODUCT	RESPONSE TO FDA REQUEST FOR INFORMATION
06-MAY-92	GENERAL CORRESP From FDA	CMC Dos Form/Potency:	DRUG PRODUCT	RESPONSE TO FDA REQUEST FOR INFORMATION RE: STATISTICAL ANALYSIS
	GENERAL CORRESP From FDA	CMC Dos Form/Potency:	DRUG SUBSTANCE	
12-MAY-92	GENERAL CORRESP To FDA	CLINICAL PRO#: INV NAME:	STUDY	
15-MAY-92	PHONE CALL	CMC Dos Form/Potency:	DRUG PRODUCT	FOLLOW-UP TO DISCUSSION ON CMC QUESTIONS

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**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      NDA      Drug Code: RP 54563      Trade Name: LOVENOX INJECTION  
 App Num: 20164      Type:      Dosage Form: SOLUTION  
 Route of Admin.: SU  
 Generic Name: enoxaparin      30 mg

COMM DATE	COMM TYPE	CONTENT Details	Name/Description	Content Comments
20-MAY-92	PHONE CALL	OTHER	N/A	DISCUSS THE CMC, STABILITY AND REVIEW STATUS
02-JUN-92	PHONE CALL	CMC Dos Form/Potency: INJECTION	DRUG PRODUCT 30 mg	TO PROVIDE FDA'S STABILITY COMMITTEE' DECISION RE: TEMPERATURE AND TIME INTERVALS
11-JUN-92	PHONE CALL	OTHER	N/A	REVIEW STATUS UPDATE
16-JUN-92	GENERAL CORRESP FROM FDA	CMC Dos Form/Potency: INJECTION	DRUG PRODUCT	THREE REQUESTS RE: MICROBIOLOGICAL PORTION OF THE APPLICATION
17-JUN-92	PHONE CALL	CMC Dos Form/Potency: INJECTION	DRUG PRODUCT 30 mg	CONFERENCE CALL TO CLARIFY FDA REQUESTS FOR ADDITIONAL STABILITY INFO IN 06-MAY-92 LETTER
18-JUN-92	PHONE CALL	OTHER	N/A	FDA ADVISED EIAR DOES NOT FULLY MEET THE CRITERIA SPELLED OUT
19-JUN-92	AMENDMENT	CMC Dos Form/Potency: INJECTION	DRUG PRODUCT N/A	RESPONSE TO 24-FEB-92 FDA LETTER PROVIDED REPRESENTATIVE TEST RESULTS & VALIDATION INFO
26-JUN-92	GENERAL CORRESP FROM FDA	OTHER	N/A	ACKNOWLEDGMENT OF RECEIPT OF 19-JUN-92 AND 24-FEB-92 AMENDMENTS. FDA EXTENED THE DUE DATE TO 26-OCT-92
29-JUN-92	AMENDMENT	CMC Dos Form/Potency: AMENDMENT	DRUG PRODUCT N/A	AMENDMENT TO A PENDING APPLICATION
14-JUL-92	PHONE CALL	PHARMACOKINETICS OTHER	N/A N/A	UPDATE OF APPLICATION STATUS FDA REQUESTED THAT WE SUBMIT SAFETY DATA UP TO AND INCLUDING 30-JUN-92
20-JUL-92	GENERAL CORRESP TO FDA	CLINICAL STUDY PRO#: INV NAME:		RESPONSE TO FDA REQUEST FOR CONFIRMATION THAT EX VIVO STUDIES ON ENO884 PATIENTS DID NOT JEOPARDIZE THE BLINDING

**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      Drug Code: RP 54563      Trade Name: LOVENOX INJECTION  
 APP Num: 20164      Type: NDA      Dosage Form: SOLUTION  
 Route of Admin.: SU  
 Generic Name: enoxaparin      30 mg

COMM DATE	COMM TYPE	CONTENT	NAME/DESCRIPTION	DETAILS	CONTENT	COMMENTS
23-JUL-92	PHONE CALL	LABEL/PRO MATERIAL	PACKAGE INSERT	FC NUM:	TO ASCERTAIN FDA'S POSITION REGARDING LOVENOX PACKAGING	
06-AUG-92	PHONE CALL	OTHER	N/A		REQUEST FOR STATISTICAL ANALYSIS	
07-AUG-92	GENERAL CORRESP TO FDA	OTHER	N/A		SELECTION OF LOVENOX AS THE TRADE NAME FOR MARKETING	
11-AUG-92	GENERAL CORRESP TO FDA	OTHER	N/A		PROVIDED CORRECTED VERSIONS OF APPENDICES XIV, XV AND XVI	
13-AUG-92	GENERAL CORRESP TO FDA	CMC Dos Form/Potency: INJECTION	DRUG PRODUCT		RESPONSE TO 16-JUN-92 FDA REQUEST FOR INFORMATION	
07-OCT-92	PHONE CALL	CMC Dos Form/Potency:	DRUG PRODUCT		PROVIDED FDA WITH A STATUS UPDATE OF DR. TEMPLE'S REVIEW	
10-NOV-92	FDA REPORT	SAFETY UPDATE	N/A		COVERING THE PERIOD 01-JAN-91 THROUGH 30-JUN-92	
20-NOV-92	GENERAL CORRESP From FDA	LABEL/PRO MATERIAL	LABEL	FC NUM:	PROVIDE DRAFT LABELING	
23-NOV-92	GENERAL CORRESP TO FDA	OTHER	N/A		RESPONSE TO APPROVAL LETTER INTENT TO FILE AN AMENDMENT	
24-NOV-92	GENERAL CORRESP TO FDA	OTHER	N/A		RESPONSE TO 20-NOV-92 REQUEST FOR ENVIRONMENTAL ASSESSMENT	
08-DEC-92	GENERAL CORRESP TO FDA	LABEL/PRO MATERIAL	LABEL	FC NUM:	RESPONSE TO 20-NOV-92 FDA REQUEST FOR INFORMATION	
11-DEC-92	PHONE CALL	OTHER	N/A		FOLLOWUP TO OUR DRAFT LABELING SUBMISSION OF 08-DEC-92	
17-DEC-92	GENERAL CORRESP TO FDA	CMC Dos Form/Potency: INJECTION	DRUG PRODUCT	30 mg	RESPONSE TO FDA REQUEST FOR INFORMATION RE: STABILITY DATA FOR 30 MG PRODUCT	
23-DEC-92	PHONE CALL	LABEL/PRO MATERIAL	PACKAGE INSERT	FC NUM:	FDA REQUESTED REFERENCE TO ANTICOAGULANT ACTIVITY BE OMITTED FROM THE DESCRIPTION SECTION OF PI	

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**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      NDA      Drug Code: RP 54563      Trade Name: LOVENOX INJECTION  
 App Num: 20164      Type: GENERAL CORRESP      Dosage Form: SOLUTION  
 Route of Admin.: SU      Generic Name: enoxaparin      30 mg

COMM DATE	COMM TYPE	CONTENT Details	Name/Description	DRUG PRODUCT	Content	Comments
24-DEC-92	GENERAL CORRESP TO FDA	CMC Dos Form/Potency:			RESPONSE TO 20-NOV-92 FDA LETTER	
30-DEC-92	PHONE CALL	OTHER	N/A		STATUS REPORT OF NDA APPROVAL	
08-JAN-93	GENERAL CORRESP TO FDA	LABEL/PRO MATERIAL FC NUM:	OTHER		RESPONSE TO FDA REQUEST FOR INFORMATION RE: FINAL PRINTED LABELING	
10-JAN-93	GENERAL CORRESP FROM FDA	CLINICAL PRO#: INV NAME:	STUDY			
14-JAN-93	GENERAL CORRESP FROM FDA	OTHER	N/A		AMENDMENT CONSIDERED MAJOR - FDA EXTENDED REVIEW DUE DATE TO 25-APR-93	
19-JAN-93	GENERAL CORRESP TO FDA	LABEL/PRO MATERIAL FC NUM:	OTHER		PROVIDED DRAFT INTRODUCTORY PROMOTIONAL INFORMATION	
19-JAN-93	PHONE CALL	OTHER	N/A		TO DISCUSS THE STATUS OF OUR APPLICATION IN GENERAL	
25-JAN-93	PHONE CALL	LABEL/PRO MATERIAL FC NUM:	OTHER		TO OBTAIN CLARIFICATION OF LABELING	
27-JAN-93	PHONE CALL	LABEL/PRO MATERIAL FC NUM:	OTHER		TO DISCUSS THE INTRODUCTORY PROMOTIONAL LAUNCH CAMPAIGN MATERIAL	
02-FEB-93	PHONE CALL	CMC Dos Form/Potency:	DRUG SUBSTANCE		NDA STATUS UPDATE	
09-FEB-93	PHONE CALL	CMC Dos Form/Potency:	DRUG SUBSTANCE		RE: STATUS OF REVIEW	
16-FEB-93	PHONE CALL	CLINICAL PRO#: INV NAME:	STUDY		TO REVIEW W/DR. VINCENT SLIGHT MODIFICATIONS TO PROTOCOL SUBMITTED 29-JAN-92	
17-FEB-93	PHONE CALL	OTHER	N/A		NDA STATUS UPDATE	
18-FEB-93	GENERAL CORRESP TO FDA	CMC Dos Form/Potency:	DRUG PRODUCT		RESPONSE TO 15-JAN-93 REQUEST RE: CONTAMINATION TESTING OF DISTILLED WATER	

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**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      Type: NDA      Drug Code: RP 54563      Trade Name: LOVENOX INJECTION  
App Num: 20164      Route of Admin.: SU      Dosage Form: SOLUTION  
Generic Name: enoxaparin      30 mg

COMM DATE	COMM TYPE	CONTENT Name/Description		Comments
		Details	Comments	
23-FEB-93	GENERAL CORRESP TO FDA	CMC Dos Form/Potency: INJECTION	DRUG PRODUCT	RESPONSE TO 15-JAN-93 FDA REQUEST FOR INFORMATION
10-MAR-93	GENERAL CORRESP FROM FDA	SAFETY UPDATE	N/A	FDA REQUESTS ADDITIONAL INFORMATION RE: 10-NOV-92 SAFETY UPDATE
29-MAR-93	GENERAL CORRESP FROM FDA	OTHER	N/A	APPROVAL LETTER
31-MAR-93	GENERAL CORRESP TO FDA	LABEL/PRO MATERIAL FC NUM:	OTHER	PROVIDED AN ADDITIONAL PIECE FOR THE INTRODUCTORY PROMOTIONAL LAUNCH CAMPAIGN INFORMATION
09-APR-93	GENERAL CORRESP FROM FDA	LABEL/PRO MATERIAL FC NUM:	OTHER	FDA COMMENTS AFTER REVIEW OF INTRODUCTORY PROMOTIONAL MATERIALS

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APPLICATION CHRONOLOGY REPORT  
Report Cover Page

Run Date: 05/05/93  
User: Ingres\_prod

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Selection Criteria

App Number: 31532

**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93 App Num: 31532 Type: IND Drug Code: RP 54563 Trade Name: TO BE ASSIGNED  
Route of Admin.: IV Dosage Form: INJECTION Generic Name: enoxaparin 100 mg/mL

COMM DATE	COMM TYPE	CONTENT	Name/Description	Details	Content	Comments
18-APR-88	ORIGINAL SUBMISSION	OTHER	N/A	N/A	ORIGINAL SUBMITTED	
19-MAY-88	GENERAL CORRESP FROM FDA	OTHER	N/A	N/A	EFFECTIVE DATE OF IND	
03-DEC-90	AMENDMENT	OTHER	N/A	N/A	PRE-NDA MEETING REQUEST	
15-JAN-91	AMENDMENT	OTHER	N/A	N/A	REQUEST FOR PRE-NDA MEETING	
17-JAN-91	AMENDMENT	OTHER	N/A	N/A	RPR NOTIFICATION OF TRANSFER OF OWNERSHIP OF IND TO RHONE-POULENC RORER 31-JUL-90	
17-JAN-91	AMENDMENT	OTHER	N/A	N/A	RPP TRANSFER OF OWNERSHIP OF IND TO RHONE-POULENC RORER ON 31-JUL-90	
06-FEB-91	GENERAL CORRESP FROM FDA	OTHER	N/A	N/A	FDA REQUEST FOR INFO TO COMPLETE THE CHANGE IN OWNERSHIP	
01-MAR-91	AMENDMENT	OTHER	N/A	N/A	CONFIRMATION OF PRE-NDA MEETING & AGENDA	
14-MAR-91	MEETING MINUTES	OTHER	N/A	N/A	PRE-NDA MEETING OVERVIEW	
25-APR-91	AMENDMENT	CLINICAL PRO#: INV NAME:	STUDY	PRO#: INV NAME:	RESPONSE TO 21-AUG-89 FDA LETTER	
	AMENDMENT	CMC Dos Form/Potency:	DRUG PRODUCT		RESPONSE TO 21-AUG-89 FDA LETTER	
15-MAY-91	AMENDMENT	OTHER	N/A	N/A	RESPONSE TO 06-FEB-91 FDA LETTER RE: ADDITIONAL INFO ON TRANSFER OF OWNERSHIP	
24-JUL-91	FDA REPORT	ANNUAL RPT	N/A	N/A	COVERING THE PERIOD 19-MAY-90 THROUGH 18-MAY-91	
14-AUG-91	AMENDMENT	CLINICAL PRO#: INV NAME:	STUDY	PRO#: INV NAME:	RESPONSE TO 21-AUG-89 FDA LETTER	
15-OCT-91	AMENDMENT	OTHER	N/A	N/A	CHANGE OF ADDRESS	

**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      Drug Code: RP 54563      Trade Name: TO BE ASSIGNED  
 APP Num: 31532      Type: IND      Dosage Form: INJECTION  
 Route of Admin.: IV  
 Generic Name: enoxaparin      100 mg/mL

COMM DATE	COMM TYPE	CONTENT	Name/Description	Details	Content	Comments
14-NOV-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	OVERDYKE,WILLIAM L RITTER,MERRILL			
21-NOV-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	COMP,PHILIP C			
22-NOV-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	GECKLER,RONALD W			
25-NOV-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	JOHNSON,GERHARD			
02-DEC-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	ZIMMERMAN,RICHARD			
09-DEC-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	FURMAN,W KIM			
11-DEC-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	KIM,HUGH C			
17-DEC-91	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	MATHEES,DONALD J			
06-JAN-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	BLAHA,J DAVID BONA,ROBERT D TROWBRIDGE,ARTHUR A			
08-JAN-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	WISE,GREGORY			

**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      Drug Code: RP 54563      Trade Name: TO BE ASSIGNED  
 APP Num: 31532      Type: IND      Dosage Form: INJECTION  
 Route of Admin.: IV      Generic Name: enoxaparin      100 mg/ml

COMM DATE	COMM TYPE	CONTENT	Name/Description	Details	Comments
15-JAN-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	547		
		INV NAME:	GONZALES, FRANCISCO WHITSETT, THOMAS		
31-JAN-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	547		
		INV NAME:	ALEDORT, LOUIS		
24-FEB-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	547		
		INV NAME:	COMEROTA, ANTHONY J		
09-MAR-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	547		
		INV NAME:	COLWELL, CLIFFORD		
10-MAR-92	AMENDMENT	CLINICAL	STUDY		BURROUGHS, M.D.; SUSAN IS LISTED AS AN INVESTIGATOR BUT NOT IN SPIN 26-MAR-92
		PRO#:	547		
		INV NAME:	BONA, ROBERT D		
17-MAR-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	547		
		INV NAME:	LYONS, ROGER M		
23-MAR-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	547		
		INV NAME:	ECONOMIDES, NICHOLAS		
01-JUL-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	YOUNG, TIMOTHY		
02-JUL-92	AMENDMENT	CLINICAL	STUDY		GEHR, TODD W.B.; AND RIPLEY, ELIZABETH D. ARE CO-INVESTIGATORS
		PRO#:	127		
		INV NAME:	SICA, DOMENIC A		
16-JUL-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	CHRISTIE, MICHAEL GUSTILLO, RAMON O'DONNELL, DENIS M		

**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      IND      Drug Code: RP 54563      Trade Name: TO BE ASSIGNED  
 APP Num: 31532      Type: Dosage Form: INJECTION  
 Route of Admin.: IV  
 Generic Name: enoxaparin      100 mg/mL

COMM DATE	COMM TYPE	CONTENT	Name/Description	Details	Content	Comments
23-JUL-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	569			
		INV NAME:	YOUNG, TIMOTHY			
23-JUL-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	569			
		INV NAME:	MERLI, GENO J			
28-JUL-92	FDA REPORT	ANNUAL RPT	N/A		COVERING THE PERIOD 19-MAY-91 THROUGH 18-MAY-92	
12-AUG-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	569			
		INV NAME:	TROWBRIDGE, ARTHUR A			
14-AUG-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	WINTERS, THOMAS F			
24-AUG-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	569			
		INV NAME:	OVERDYKE, WILLIAM L			
26-AUG-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	569			
		INV NAME:	JOHNSON, WILLIAM M			
02-SEP-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	569			
		INV NAME:	TREMAINE, M DAVID			
08-SEP-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	547			
		INV NAME:	MANNAL, RICHARD			
08-SEP-92	AMENDMENT	CLINICAL	STUDY		US00468 CARLTON SAVORY NOT IN SPIN	
		PRO#:	569			
		INV NAME:	HAIRE, WILLIAM D			
22-SEP-92	AMENDMENT	CLINICAL	STUDY			
		PRO#:	124			
		INV NAME:	FURMAN, W KIM			

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**APPLICATION CHRONOLOGY REPORT**

Run Date: 05/05/93      App Num: 31532      Type: IND      Drug Code: RP 54563      Trade Name: TO BE ASSIGNED  
Route of Admin.: IV      Dosage Form: INJECTION      Generic Name: enoxaparin      100 mg/mL

COMM DATE	COMM TYPE	CONTENT	Details	Name/Description	Comments
07-OCT-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	STULBERG, BERNARD		
08-OCT-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	FURMAN, W KIM		
20-OCT-92	FDA REPORT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	BERNASEK, THOMAS L		
11-NOV-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:			
07-DEC-92	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	OHAR, JILL		
21-DEC-92	AMENDMENT	INVEST IND	N/A		
12-JAN-93	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	FISHER, DAVID A		
19-JAN-93	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	WINTERS, THOMAS F		
26-JAN-93	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	GECKLER, RONALD W		
			RITTER, MERRILL		
11-FEB-93	AMENDMENT	CLINICAL	STUDY		
		PRO#:	569		
		INV NAME:	LEDES, CLAUDE P		

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Run Date: 05/05/93 Type: IND Drug Code: RP 54563 Trade Name: TO BE ASSIGNED  
APP Num: 31532 Route: IV Dosage Form: INJECTION  
Route of Admin.: IV Generic Name: enoxaparin 100 mg /mL

COMM DATE	COMM TYPE	CONTENT Details	Comments
01-MAR-93	AMENDMENT	CLINICAL PRO#: 569 INV NAME: BERNASEK, THOMAS L WHITSETT, THOMAS	STUDY

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE

<b>DECLARATION PURSUANT TO 37 C.F.R. 1.740(b)</b>		Docket Number: <b>1247/1</b>	
Application Number <b>726,178</b>	Filing Date <b>4/23/85</b>	Examiner <b>Johnie R. Brown</b>	Art Unit
Patent Number <b>4,692,435</b>	Issue Date <b>September 8, 1987</b>		
<b>Invention Title</b> Mucopolysaccharide Composition Having a Regulatory Action on Coagulation, Medicament Containing Same and Process of Preparation		<b>Inventor(s)</b> Lormeau et al.	

- (1) I am an official of Choay S.A., authorized to obligate the corporation.
- (2) I have reviewed and understand the contents of the application being submitted pursuant to this section.
- (3) I believe U.S. patent 4,692,435 (the "435 patent") is subject to extension pursuant to 37 C.F.R. § 1.710.
- (4) I believe an extension of the length claimed is justified under 35 U.S.C. 156 and the applicable regulations.
- (5) I believe the '435 patent meets the conditions for extension of the term of a patent as set forth in § 1.720.
- (6) Choay S.A. is the assignee and owner of 100% interest in the '435 patent.
- (6) I declare the foregoing is true and correct, under penalty of perjury.

Signed: Jacques SAMPRE

Title: Président

24/05/1993



exhibit

**UNITED STATES DEPARTMENT OF COMMERCE**  
**Patent and Trademark Office**

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## **MAINTENANCE FEE STATEMENT**

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. **TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).**

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. **THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.**

TM IBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	4,692,435	173	830	----	06/726,178	09/06/87	04/23/85	04	NO	PAID